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ORIGINAL ARTICLES

CLINICAL AND EXPERIMENTAL STUDIES ON THE EFFECT OF CORTICOTROPIN AND STEROID DRUGS ON BILIRUBINEMIA

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Jaundice in patients with acute hepatitis often clears rapidly during steroid therapy (Ducci and Motlis[3]), especially when the illness is associated with clinical, biochemical or histologic features suggesting biliary obstruction (Sborov *et al.*[8], Solem and Olsen[11], Summerskill and Jones[13]). However, the effect is variable (Sborov *et al.*[8]), and as some reduction in concentration of serum bilirubin may follow treatment in patients with extrahepatic obstruction of the bile ducts (Chalmers *et al.*[2], Katz, Ducci and Alessandri[4]), the specificity and nature of the response of bilirubinemia to steroids remain uncertain.

In this paper the effect of steroids or corticotropin on bilirubinemia and on hepatic function is reported in patients with jaundice having obstructive features. Experimental studies of possible mechanisms of reduction of hyperbilirubinemia have been carried out in dogs by assessing the effect of cortisone on bile secretion and, in animals with biliary obstruction, on red-blood-cell survival and serum-bilirubin concentration.

Clinical Studies. MATERIALS AND METHODS. Twenty-two patients, aged between 10 and 74 years, were studied. All had clinical and biochemical findings suggesting jaundice of an obstructive type with serum-bilirubin con-

*On assignment to the Mayo Foundation. The contents of this article are the personal views of the authors and are not to be construed as official Air Force policy or as Air Force endorsement of any commercial product described.

centrations of more than 10 mg. per 100 ml. for at least 2 weeks and an elevated serum alkaline phosphatase activity, values being more than twice normal in all but 6. Itching was a prominent symptom in 18 patients. Seven patients were subsequently proved to have hepatitis, 5 had cirrhosis of uncertain etiology and the remainder had carcinoma of the ampulla, bile ducts or pancreas.

mg. daily by mouth), 5 were given corticotropin (60 units daily by injection), 2 had cortisone (200 to 300 mg. daily) by mouth, and one was treated with triamcinolone (12 mg. daily).

Results (Fig. 1 and Table 1). Of patients subsequently shown to have hepatitis, variable increases in con-

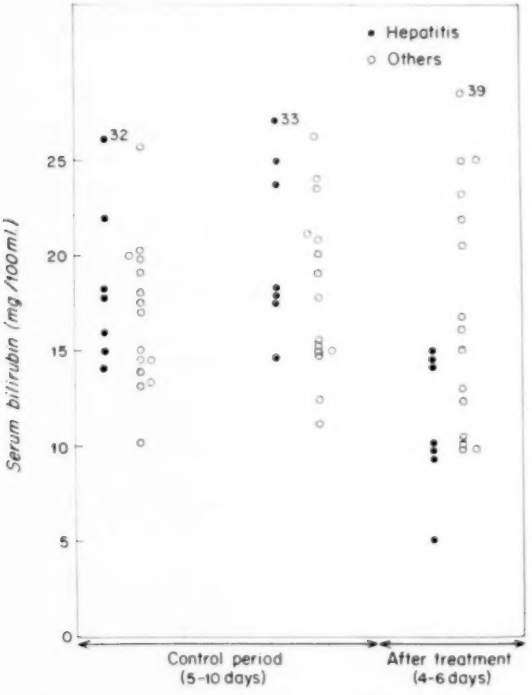

Fig. 1.—Concentration of serum bilirubin before and after treatment with corticotropin or steroid drugs in patients with jaundice.

Serum-bilirubin content was estimated by standard modifications of the methods of van den Bergh or Evelyn and Malloy, and the activity of serum glutamic-oxalacetic transaminase (SGOT) by a modification of Karmen's method (Siekert and Fleisher[10]). Corticotropin or steroid drugs were given after at least two determinations of serum-bilirubin concentration had been made before and after a control period of 5 or more days. The drugs were administered daily for 5 days or longer, after which the concentration of serum bilirubin was again determined. Fourteen patients received prednisone (30 to 60

centration of serum bilirubin occurred in the majority during the control periods, and in no patient was there a decline of more than 2 mg. per 100 ml. When steroids or corticotropin were given, rapid improvement in respect to jaundice occurred within 4 to 6 days in every patient, final levels in all instances being 40% or more below the initial reading. Continued drug treatment was accompanied by a sustained

reduction of serum-bilirubin concentration to normal.

Patients with extrahepatic obstruction or cirrhosis had variable responses. In 6 patients some decrease in concentration of serum bilirubin occurred, but the greatest decrease was only 35%. Seven patients became more icteric despite treatment, the greatest increase being 35% above the initial value at the end of 5 days. Further determinations of serum-bilirubin concentration were carried out 5 more days after drug administration in 9 patients.

TABLE 1.—CONCENTRATION OF SERUM BILIRUBIN (SB) IN MG./100 ML. AND ACTIVITY OF SERUM GLUTAMIC-OXALACETIC TRANSAMINASE (SGOT) IN μM/ML./HR. AT 38° C. BEFORE AND AFTER TREATMENT WITH PREDNISONE OR CORTISONE

	Before		*After*	
	SB	*SGOT**	*SB*	*SGOT*
Case 1	7.4	8.0	12.2	4.8
2	8.9	25.7	14.4	3.0
3	9.9	24.9	12.9	3.0
4	21.0	84.7	21.9	20.6
Dog 1	13.2	3.9	14.2	4.7
2	8.5	8.6	9.5	11.3
3	7.8	4.7	10.0	11.7
4	10.0	9.8	9.2	5.5

*Normal mean values with standard errors of means: humans 0.91 ± 0.02 μM/ml./hr.; dogs, 1.05 ± 0.03.

Jaundice increased in 5, lessened in 2, and was unchanged in the other patients.

In patients with hepatitis the decrease in concentration of serum bilirubin during treatment was accompanied by return toward normal of the results of chemical tests of hepatic function, including serum-transaminase activity, and by simultaneous improvement in the general condition. The latter was best demonstrated by increase in appetite. Caloric intake for the 4-day periods before and after treatment increased strikingly in each of 4 patients, the mean change being from 740 to 1700 calories daily.

Changes in concentration of serum bilirubin during treatment were comparably inconspicuous in patients with cirrhosis and extrahepatic obstruction. However, in 4 patients with cirrhosis in whom serum-transaminase activity was measured before and after treatment with prednisone for 5 days, values for it decreased, although bilirubinemia increased (see table).

Experimental Studies. MATERIALS AND METHODS. Experiments were performed on young adult male dogs maintained on standard kennel rations with an unrestricted fluid intake. Serum-bilirubin concentration and transaminase activity were measured as described earlier. The following preparations were employed: 1. External biliary fistulas: These were produced in 4 dogs after cholecystectomy with ligation and division of the cystic and common ducts and intubation of the latter for the fistula. Twenty-four-hour collections of bile from the fistulas were measured daily (but not readministered to the animals), and the concentration and total content of bilirubin in each specimen were determined. Measurements were made during each of three 5-day periods; namely, before drug administration, during administration of one intramuscular dose of cortisone (50 mg.) daily, and after cortisone administration. Food and fluid intake were comparable during these periods. 2. Biliary obstruction and erythrocyte survival: Biliary obstruction was produced in 6 dogs by cholecystectomy with ligation of the cystic duct and section and ligation of the common duct. After variable periods during which bilirubinemia reached fairly constant levels, as indicated by consecutive values for serum bilirubin that were within 1 mg. per 100 ml. of each other on successive weeks, each animal received an intramuscular injection of 50 mg. of cortisone daily for 5 days. At the end of this period and again after a further 5 days, the concentration of serum bilirubin was determined. In 3 dogs, 2 with biliary obstruction and one normal animal, erythrocyte survival time was measured before, during and after cortisone administration, by means of red blood cells labeled with radiochromium (Cr_{51}) (Owen[6]).

Results. 1. EFFECT OF CORTISONE ON HYPERBILIRUBINEMIA ASSOCIATED WITH BILIARY OBSTRUCTION (FIG. 2). The concentration of serum bilirubin was increased in every animal (6.5 to 12.0 mg. per 100 ml.) at the end of the control period, following stabilization of the concentration 3 to 4 weeks after ligation of the hepatic ducts. The administration of cortisone acetate had little effect, being followed by a slight decrease in the concentration of serum bilirubin (1.5 mg. per 100 ml.) in one animal, but by increases of 0.5 to 1.3 mg. per 100 ml. in the other 3. Body weight decreased or remained stable. After drug administration the concentration of serum bilirubin showed little change in 3 animals, but it continued to rise by a further 1.8 mg. per 100 ml. in the dog with the greatest increase in hyperbilirubinemia during cortisone administration. Values for serum transaminase showed no consistent or striking change (Table 1).

2. ERYTHROCYTE SURVIVAL TIME WITH BILIARY OBSTRUCTION (FIG. 3). Erythrocyte survival time in animals with jaundice due to biliary obstruction was comparable to that in the normal animal. During cortisone administration no change in erythrocyte survival time was found in any animal.

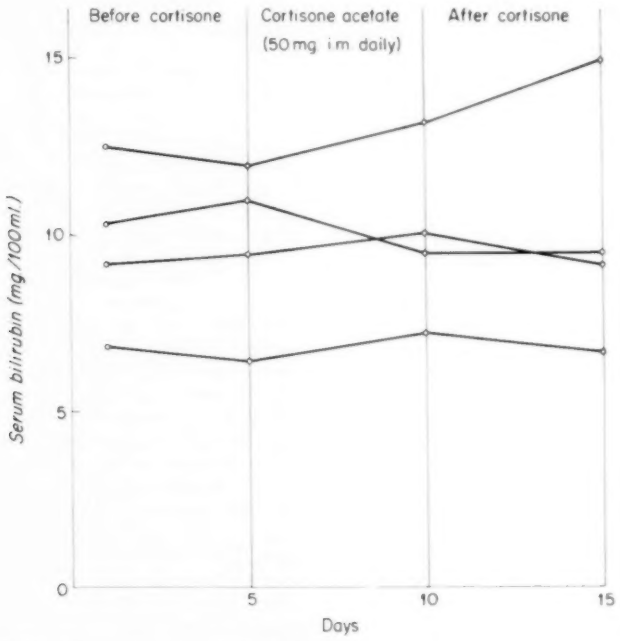

Fig. 2.—Effect of cortisone on hyperbilirubinemia in dogs with biliary obstruction.

3. BILIARY FISTULAS (FIG. 4). Mean daily volume of bile was increased in 3 of 4 animals during the administration of cortisone and reverted toward earlier values after the drug was withdrawn. However, the excretion of bilirubin was not altered proportionately, remaining unchanged in one animal, decreasing in another, and increasing in 2 animals.

Discussion. The clinical findings sup-

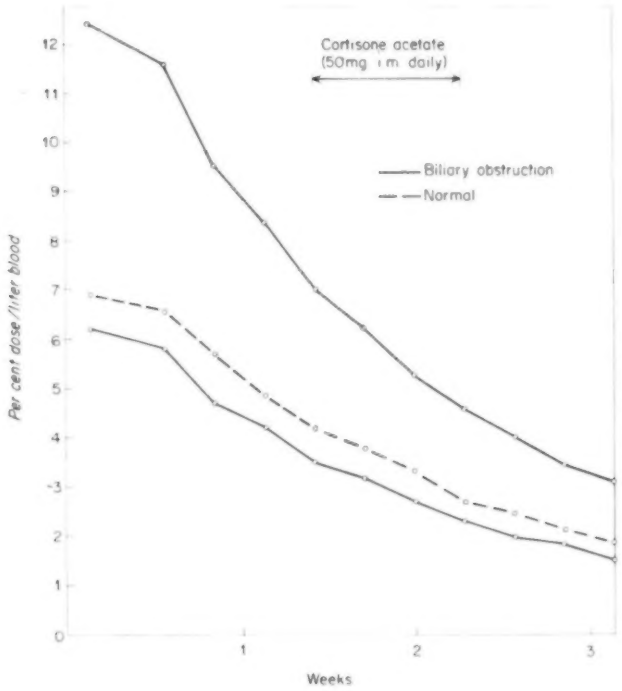

Fig. 3.—Survival of erythrocytes and concentration of serum bilirubin in relation to administration of cortisone. The ordinate represents the percentage of radiochromium-labeled red blood cells per liter of blood.

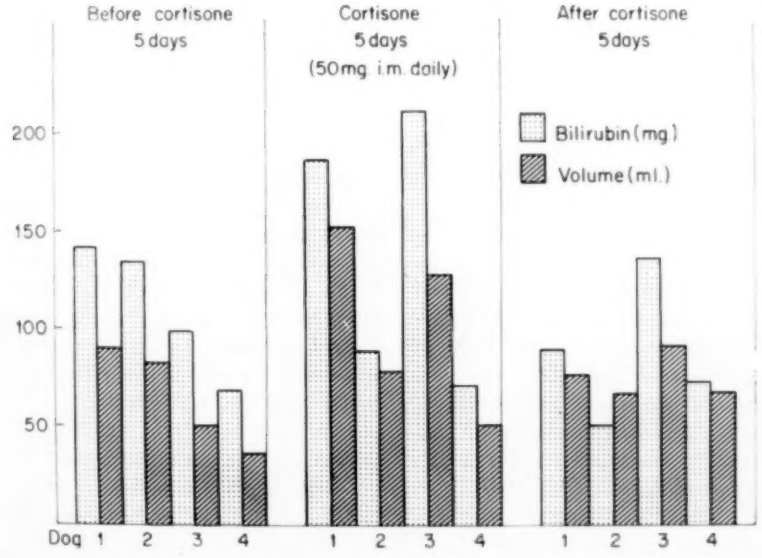

Fig. 4.—Relation of mean 24-hour volume and bilirubin content of bile in dogs with biliary fistulas to administration of cortisone.

port the view that therapy with steroids or corticotropin is effective in lowering the concentration of serum bilirubin in patients with acute viral hepatitis when associated with features suggesting biliary obstruction, and that the drugs can often be usefully employed under such circumstances as a diagnostic test (Sborov *et al.*[8], Solem and Olsen[11], Summerskill and Jones[13]). A fall in serum-bilirubin concentration of 40% or more of the initial value occurred in 4 to 6 days in all patients with hepatitis, thus differentiating them from patients with similar clinical and biochemical findings, who were later shown to have cirrhosis or obstructive jaundice due to neoplastic disease.

However, the place of these drugs in the treatment of acute and chronic disease of the liver is controversial (Summerskill[12]) and awaits definition of their specific action in patients with jaundice. Studies in animals with ligated hepatic ducts indicated that cortisone had no direct effect on hyperbilirubinemia or serum-transaminase activity in the absence of hepatic disease and, as erythrocyte survival was not influenced in such animals, that the action of steroids in reducing jaundice appears to be independent of a decrease in bilirubin formation through retardation of red-cell breakdown. The failure to gain weight significantly in either animals or patients during the administration of corticotropin or steroid drugs suggests that dilution due to fluid retention is not an important factor in reducing the concentration of serum bilirubin. Although dogs with biliary fistulas excreted rather larger volumes of bile during cortisone administration, in accordance with earlier observations on humans (Patterson *et al.*[7]), the bilirubin content did not change proportionately, so a specific action that increases bilirubin excretion cannot be postulated. These findings suggest, therefore, that changes reported in concentration of serum bilirubin during treatment in patients with bile-duct obstruction reflect coincidental fluctuations in the depth of jaundice resulting from increased renal clearance, greater tissue solubility of bilirubin conjugates, or metabolism to compounds giving no diazo reaction.

The lessening of jaundice in patients with hepatitis indicates a direct effect of steroids and corticotropin on the liver. Although prednisone may increase the conjugation of bilirubin to glucuronides (Auricchio, Martucci and Mormone[1]), the more constant reduction of jaundice when obstructive features are associated with hepatitis (other than that induced by drugs [Summerskill and Jones[13]]) indicates an additional effect on mechanisms governing the excretion of conjugated bilirubin from the hepatic cell. The degree to which this reflects an over-all improvement in hepatic function is uncertain, but continued therapy in patients with hepatitis was associated with progressive clearing of jaundice and improvement in appetite and other clinical and biochemical estimates of hepatic function, although it has been suggested that the histologic changes in the liver are affected less rapidly (Shaldon and Sherlock[9]).

The failure of the serum-bilirubin concentration to fall consistently in response to treatment in patients with cirrhosis indicates that the capacity of the hepatic cell to excrete conjugated bilirubin improves less rapidly in chronic than in acute hepatic disease after the administration of steroid drugs. However, the striking reduction in serum-transaminase activity in our patients with cirrhosis presumably reflects a decrease in hepatic-cell necrosis in response to treatment. The disparity between changes in serum bili-

rubin and transaminase activity was very great and it is possible that lessening of jaundice might have followed if therapy had been continued for a longer period. O'Brien, Goble and Mackay[5] found that serum-transaminase activity fell rapidly after cortisone therapy in patients classified as having active chronic hepatitis and similar to those studied by us, but not when cirrhosis was associated with alcoholism or hemochromatosis. The more striking response to steroids in patients with acute or chronic hepatitis, therefore, supports the concept of a specific action of these drugs in hepatic disease associated with autoimmune mechanisms (O'Brien, Goble and Mackay[5]) and would thus explain some of the equivocal responses reported in diseases of the liver presumed to be of different causes.

Summary. 1. Following the administration of steroids or corticotropin, the greater fall in concentration of serum bilirubin in patients with hepatitis having obstructive features often allows a differentiation to be made from jaundice due to cirrhosis or extrahepatic obstruction. In patients with acute hepatitis, reduction in bilirubinemia was associated with a striking increase in caloric intake and improvement in respect to chemical tests of hepatic function. Although no consistent change in bilirubinemia occurred in patients with cirrhosis during a 5 to 10-day period of treatment, serum-transaminase activity decreased greatly.

2. Studies in dogs with jaundice following ligation of the bile duct showed that cortisone had no effect on the concentration of serum bilirubin or on erythrocyte survival time. In dogs with biliary fistulas that were receiving cortisone, the volume of bile excreted increased, but the bilirubin content was little changed.

3. It is concluded that steroids or corticotropin influences jaundice only by an effect on hepatic function. Evidence is put forward to support the view that this may be a specific action limited to hepatic disease associated with autoimmune factors.

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SUMMARIO IN INTERLINGUA

Studios Clinic e Experimental in Re le Effecto de Corticotropina e Drogas Steroide in Bilirubinemia

1. Post le administration de corticotropina o de steroides, le plus marcate declino del concentration seral de bilirubina que occurre in patientes con hepatitis

qui ha aspectos obstructive permitte frequentemente le establimento de un differentiation ab jalnessa causate per cirrhosis o obstruction extrahepatic. In patientes con hepatitis acute, le reduction del bilirubinema esseva associate con un frappante augmento del ingestion caloric e con meliorationes con respecto a tests chimic del function del hepate. Ben que nulle uniforme alteration del bilirubinemia occurreva in patientes con cirrhosis in le curso de un periodo de 5 a 10 dies de tractamento, le activitate de transaminase seral declinava marcatemente.

2. Studios in canes con jalnessa post ligation del vias biliari monstrava que cortisona habeva nulle effecto super le concentration seral de bilirubina o super le longevitate del erythrocytos. In canes con fistulas biliari que esseva tractate con cortisona, le volumine del excernite bile esseva augmentate, sed le contento de bilirubina esseva pauco alterate.

3. Es concludite que steroides o corticotropina exerce un influentia super jalnessa solmente per un effecto in le function hepatic. Es presentate datos que supporta le opinion que isto es possibilemente un action specific que es limitate a morbo hepatic associate con factores autoimmun.

NOTES ON TECHNIQUES OF INTRAMUSCULAR INJECTION

THE AVOIDANCE OF NEEDLESS PAIN AND MORBIDITY

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(From the Medical Service, Veterans Administration Hospital, Topeka, Kansas)

Intramuscular injections, like many other technical procedures, were originally administered by physicians but have been largely taken over by the nursing profession. This is as it should be, for the physician would be greatly limited in his effectiveness if he were required to administer these himself. Being no longer familiar with the technical details of the intramuscular injection, physicians may be inclined to treat them perfunctorily. Such an attitude, innocently acquired by nurses, may lead to improper practices of that technique, with resulting unnecessary pain and morbidity to patients. Patients rarely complain of the pain inflicted, for they tend to assume such suffering to be a necessary part of the procedure.

Prior to the introduction in 1945 of penicillin therapy, physicians were well taught and practiced in the technique of intramuscular injection by virtue of their experience in the syphilis clinics. The injection of irritant bismuth or mercury salts required a meticulous technique to avoid disaster. Such a technique was described by Stokes[8], whose comprehensive textbook of syphilology devoted 7 pages to a discussion of the choice and care of needles, 8 pages to the technique of intramuscular injection, and 4 pages to the prevention and treatment of complications of intramuscular injections (Stokes, Beerman and Ingraham[9]). It is surprising how scant are the descriptions found in textbooks of nursing.

Being employed full time in a hospital, I have often observed errors and omissions in the technique of intramuscular injections administered by nurses. In my experience, nurses have taken kindly to correction, and have many times told me that their teaching had not included all such details of technique. To test the generality of the truth of such a statement, a questionnaire (Table 1) was submitted to the 108 nurses employed at this hospital. The 101 replies which were received proved disturbing in their near universality of error and omission and in their reports of the inadequacy of their teaching. This is probably a representative experience, since our nurses were educated in 55 different nursing schools located in 23 states.

Site of injection (Fig. 1-2). The upper outer quadrant of the buttock is the site of choice in the traditional method of intramuscular injection. This quadrant allows a maximal thickness of muscle to receive the injected material, so that it may be deeply injected. It avoids the blood vessels and nerves which are present in profusion in the upper inner quadrant, and the great sciatic nerve in the lower inner quadrant. No other quadrant of the buttock is acceptable. Injections into the deltoid or other muscles is less satisfactory, because of the thinness

and greater sensitivity of these muscles, and the proximity of vulnerable nerves.

Of 101 nurses reporting, 92 replied satisfactorily that they always used the upper outer quadrant, but 9 did not always do so (even by intention). Five of these 9 nurses were among 18 who claimed to have been taught that the use of other quadrants of the buttock was admissible.

The greatest hazard to which the intramuscular injection into the buttock is subject is injection into or near the sciatic nerve in the lower inner quadrant. Shaffer[7] made a careful study of the anatomy of the gluteal muscles and the distribution of intramuscularly injected substances. He found that injections into the lower central portion of the buttock endangered the greater

TABLE 1.—QUESTIONNAIRE SUBMITTED TO NURSES, WITH TABULATION OF REPLIES RECEIVED

TO ALL NURSES:
Your cooperation in answering this questionnaire is requested in order to evaluate teaching practices in regard to the technique of intramuscular injection into the buttock. You need not sign this questionnaire.

1a. In administering intramuscular injections into the buttock, do you use the upper outer quadrant?
Always 92 Not always 9

1b. Were you taught the permissibility of use of other quadrants?
Yes 18 No 82 Unanswered 1

2a. Do you administer such injections in the prone position?
Always 45 Not always 56

2b. Were you originally taught to insist upon this position only?
Yes 47 No 53 Unanswered 1

3a. Do you ask the patient to "toe in?"
Always 12 Not always 84 Unanswered or questionmarked 5

3b. Were you originally taught to have patients "toe in?"
Yes 10 No 86 Unanswered 5

4a. Do you draw down the buttock before inserting the needle?
Always 58 Not always 43

4b. Were you originally taught to draw down the buttock?
Yes 46 No 53 Unanswered or questionmarked 2

5a. Do you aspirate for blood prior to injection?
Always 97 Not always 4

5b. Were you originally taught the necessity for such aspiration?
Yes 94 No 7

6a. Do you massage the injection site after injection?
Always 72 Not always 29

6b. Were you originally taught to massage the injection site?
Yes 81 No 14 Unanswered or "sometimes" 5

7a. In your experience, have any patients ever complained of pain or numbness in the foot after injection?
Yes 7 No 93 Questionmarked 1

7b. In your experience, have any patients developed painful lumps persisting for days after injection?
Yes 48 No 51 Questionmarked 2

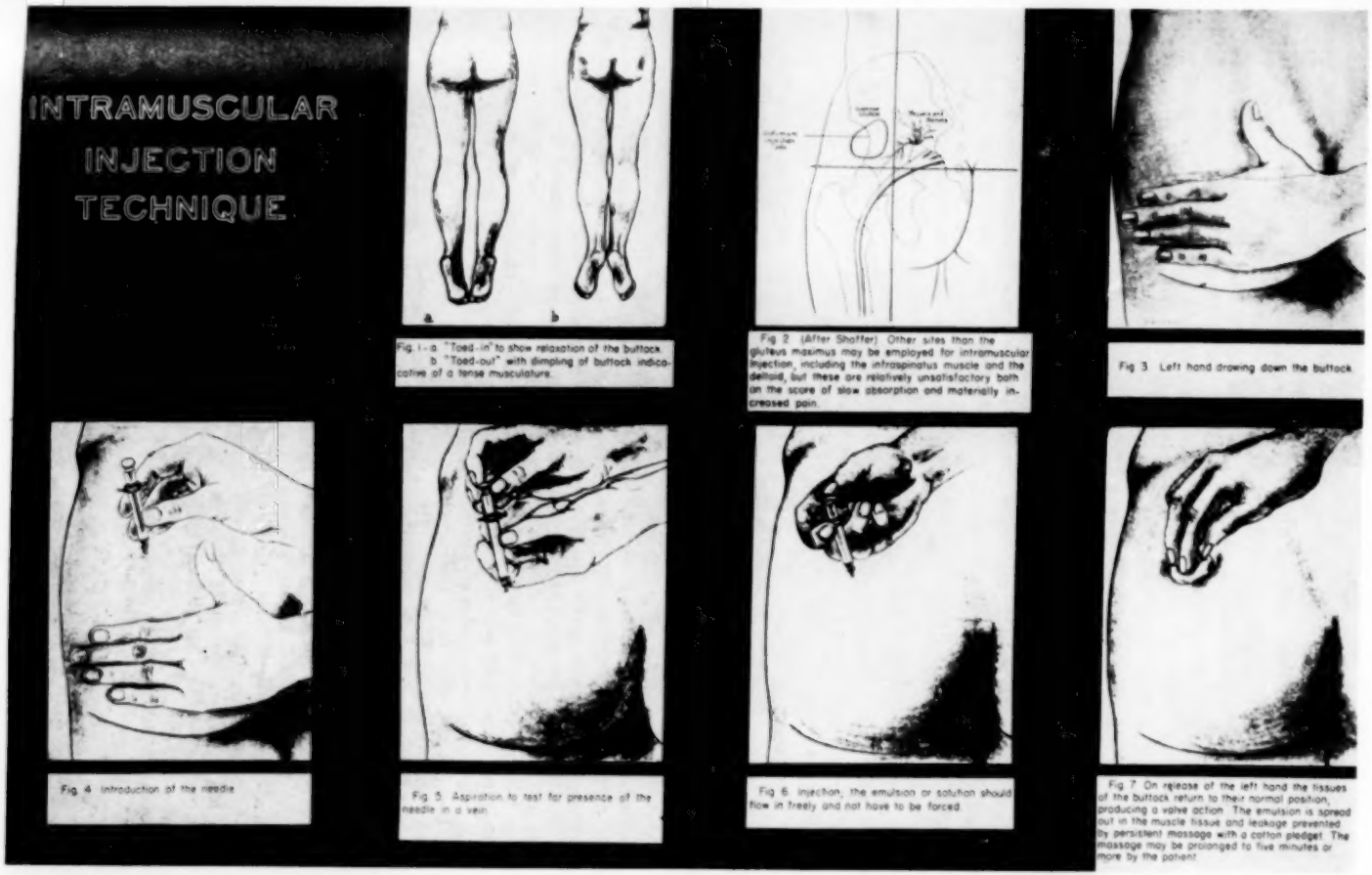
INTRAMUSCULAR
INJECTION
TECHNIQUE
Fig. 1—a. "Toed-in" to show relaxation of the buttock. b "Toed-out" with dimpling of buttock indicative of a tense musculature.
Fig. 2 (After Shaffer). Other sites than the gluteus maximus may be employed for intramuscular injection, including the infraspinatus muscle and the deltoid, but these are relatively unsatisfactory both on the score of slow absorption and materially increased pain.
Fig. 3. Left hand drawing down the buttock.
Fig. 4. Introduction of the needle.
Fig. 5. Aspiration to test for presence of the needle in a vein.
Fig. 6. Injection; the emulsion or solution should flow in freely and not have to be forced.
Fig. 7. On release of the left hand the tissues of the buttock return to their normal position, producing a valve action. The emulsion is spread out in the muscle tissue and leakage prevented by persistent massage with a cotton pledget. The massage may be prolonged to five minutes or more by the patient.

Fig. 1.—Steps in the traditional technique of intramuscular injection into the posterior gluteal muscles. (Reproduced from Stokes, Beerman and Ingraham[9] with permission of the authors and publisher.)

sciatic nerve if they extended through the gluteus maximus muscle. The more common and temporary sciatic-like pain extending down the back of the thigh he attributed to irritation of the small sciatic nerve which lies immediately over the great sciatic nerve in this area. Injected fluid was well dispersed within the first few minutes, assuming a curved linear distribution within the gluteus maximus, being closely confined to the coarse fasciculi into which this muscle is divided.

The frequency with which injection into or near the sciatic nerves occurs may be gauged by the replies to item 7a in the questionnaire, in which 7 or 8% of the nurses reported having encountered pain or numbness in the foot after injections. Two brief case reports illustrate the morbidity produced by this hazard:

Case Reports. CASE 1. A 37-year-old man was admitted October 2, 1957, to the Veterans Administration Hospital in Topeka, complaining of pain and weakness in the left leg of 5 weeks' duration. In August, he had been hospitalized elsewhere for one week because of nervous tension following a bout of alcoholism. He had been given an intramuscular injection of Thorazine in the left buttock. Immediately following the injection, the left foot became numb, and he fell to the floor. Since then, there had persisted severe pain and a burning sensation of the anterior aspect of the left leg and foot. Diathermy and vitamin therapies had been used. The weakness had begun to improve at the time of admission to the Veterans Hospital.

The left Achilles tendon reflex was absent, and there was inability to dorsiflex the left foot and toes. Severe weakness was demonstrable of the flexor hallucis longus, tibialis posterior, and gastrocnemius muscles. Sensory loss was present over the lateral aspect of the left leg and the dorsum of the left foot, corresponding to the distribution of the peroneal nerve.

Treatment consisted of more vitamins, an extension splint for the foot, physiotherapy, adrenal steroid therapy, and hypnotherapy to aid in relaxation of secondary muscle spasm in the leg. By the end of November, considerable improvement had occurred in motor power, but subjective burning and pain continued thereafter, finally disappearing at the end of April, at which time the patient was discharged from the hospital. Examination then showed intact Achilles reflex, and no remaining sensory loss.

CASE 2. A 59-year-old man was admitted July 14, 1959, to the Veterans Administration Hospital in Topeka, complaining of numbness and pain in the right foot, of 2 months' duration. He had been hospitalized elsewhere April 9 to May 8, 1959, having entered because of hemorrhoidal bleeding but kept hospitalized for neurologic study following the occurrence of a grand mal seizure. During that hospitalization, he had received many intramuscular injections, usually in the right buttock. These were given, according to the patient's story, in lying or standing positions, and in outer or inner aspects of the buttock. Following some of these injections, the patient developed lumpiness and tenderness in the lower inner quadrant of the buttock, pains down his right leg, and numbness of the right foot.

Chronic disabling pain in the right foot began May 5, three days before discharge from the hospital. In the next 2 months, the patient was attended by his home physician. Initially, pain involved the plantar area of the foot, and extended to the calf of the leg, posterior thigh and hip. Improvement occurred during the second month. At the time of rehospitalization, pain and numbness were residual only in the foot. Tingling occurred on walking or on contact against the foot. Pain was not accentuated by walking, but tended to be worse during the night. There were no sensations of temperature change.

Examination revealed weakness of the right Achilles tendon reflex, but no muscular atrophy was detected. Pinprick sensation was intact, but resulted in marked tingling and muscular movements of the foot. There was absence of touch sensation on the medial aspect of the base of the right great toe. The right foot was warmer than the left, despite a weaker dorsalis pedis pulse. The left leg showed no sensory disturbances.

During his hospitalization, lasting 11 days, the patient continued to improve, and he was discharged with reassurance of continued improvement.

POSITION OF THE PATIENT (Fig. 1-1). The intramuscular injection should be given with the patient in the prone position, toes facing inward and heels

outward. Pointing the toes inward and heels outward causes gluteal muscle relaxation, automatically maintained by the patient. This position is preferred to the position designed to achieve relaxation which is advocated by some authors (Dutton and Lake[1]), in which the patient lies prone with the head turned to one side, the arms hanging over the sides of the treatment table or bed, and the knees bent with a pillow under the shins supporting the legs.

innervation of muscle consists predominantly of pressure-sensitive nerve fibers rather than pain-sensitive fibers. The contracted muscle may fail to accommodate the injected solution, tending to force it back along the track of the needle and into the subcutaneous tissue. The dangers of subcutaneous leakage of injected solution are discussed below in relation to length of needle.

Only 45 of 101 nurses reporting stated that they always used the prone position for intramuscular injections,

Fig. 2.—Painful inflammatory induration ("sterile abscesses") of the subcutaneous tissues of both buttocks following multiple injections over a period of one week. Breakdown of skin on the right side has resulted in seepage and crusting of exudate.

Standing or sitting positions have been described, but add increased hazards. The patient may make a sudden jerking movement at the time of painful entry of the needle. My only personal experience of a broken needle occurred during injection in the sitting position, when the patient made a sudden rising movement on insertion of the needle; incision and probing under the fluoroscope were necessary.

In the standing position, proper relaxation of the gluteus muscle may not be achieved, or the muscle may be sharply contracted on entry of the needle. Injection into a contracted muscle is painful, because the sensory

and only 47 admitted having been taught to insist upon this position only. Of the 56 nurses who did not always use the prone position, 12 had been taught to insist upon that position, indicating the frequency of lapses from properly taught procedure when the subsequent environment proves indifferent.

Only 12 of the 101 nurses always asked patients to "toe in." Among the 89 who did not do so, were 5 unanswered or questionmarked replies and 3 written responses reading "never!" Such responses probably indicate total unfamiliarity with this part of the technique.

LENGTH OF NEEDLE. The needle should be long enough to reach well into the belly of the muscle. Otherwise, there is danger of injection into deep subcutaneous tissue. This point was not included in the questionnaire, but I have repeatedly discovered nurses using needles of inadequate length. Such instances usually come to my notice after a patient has complained of persistent painful deep subcutaneous nodules (Fig. 2).

A needle of one inch length is never adequate in any but emaciated adults, while a needle of 3 or even 4 inches length may be required in an extremely obese person. Usually, a needle of 1½, 2, or 2½ inches length will suffice.

Shaffer[7] produced radiographs of subcutaneous leakage following intramuscular injections of iodized oil, finding that such leakage could be readily prevented by using a needle of small gauge, by drawing aside the skin and subcutaneous tissue of the buttock prior to injection, by making the injection well within the muscle, by leaving the needle in place a few moments on completion of injection, and by applying pressure with a cotton pledget immediately on its withdrawal. On the other hand, subcutaneous leakage could be deliberately produced by using a

Fig. 3.—Radiograph showing subcutaneous leakage and nodule formation following injection of iodized oil into the gluteus maximus muscle. (Reproduced from Shaffer[7] with permission of the author and publisher.)

Many medications require intramuscular injection solely because of the greater pain caused by them if injected subcutaneously, due to the greater subcutaneous sensory innervation. Other medications are injected intramuscularly because of the need for more rapid absorption. Slower absorption in the subcutaneous tissue not only defeats the requirement of more rapid systemic effect, but also allows greater tissue reaction to the irritant qualities of the longer persisting medication, leading to persistent painful encapsulated nodules or "sterile abscesses."

large (20 gauge) needle and withdrawing it quickly on completion of the injection, without making any effort to secure the valve action of the skin by pulling it to one side prior to injection. The Roentgen-ray film clearly showed the point of injection slightly within the gluteus maximus muscle, the line of leakage with its collection into a ball-like deposit in the fatty superficial fascia, and extension of the jagged line of leakage to the skin surface (Fig. 3).

To the question whether in their experience any patients had developed painful lumps persisting for days after injection, 48 nurses replied in the affirmative, 51 in the negative, and 2 with questionmarks. This is surely an unnecessarily high frequency of such experiences. Six of the nurses added that such experiences had followed repeated injections, and one wrote that they occurred "frequently." Three nurses added that such painful lumps had followed certain medications. Thorazine, antibiotics, streptomycin, and heparin were mentioned specifically. What these medications share in common is that they are in common use in this hospital. One nurse added that the painful lumps were due to "poor circulation." Thus do faulty techniques persist, while their unhappy effects are projected onto other causes. "Some persons," one of my teachers once remarked, "can make the same mistake for 20 years, and call it 'experience.'"

INTRODUCTION OF THE NEEDLE (Fig. 1-3, 4). The important point in introducing the needle is the maneuver of retracting the skin and subcutaneous tissues. Theoretically, at least, when the needle is later withdrawn the return of these superficial tissues to their normal positions serves to break the direct needle track, thus decreasing the likelihood or quantity of subcutaneous seepage of injected material from within the muscle belly. A more important function, perhaps, is served by the tactile knowledge thus imparted to the nurse of the approximate location in depth of the firmer muscular tissue underlying the movable subcutaneous mass.

Only 58 nurses out of 101 stated they always performed this maneuver. Of the 43 who did not always do so, one wrote she made the skin taut instead, apparently confusing this maneuver with the technique used in subcutaneous injection. Forty-six remembered being taught this maneuver, while 53 were not so taught, and 2 unanswered or questionmarked replies were received. One nurse reported she had been taught instead to "draw down on a thin patient, pinch up on a heavy patient," which makes no apparent sense. In fact, the "pinching up" maneuver would be likely to increase the hazard of injecting near the sciatic nerve.

ASPIRATION (Fig. 1-5). This maneuver is designed to test for accidental entry of the needle into a bloodvessel, and is necessary to avoid intravenous (or intra-arterial) instead of intramuscular injection. Many medications designed for intramuscular use would be dangerous if introduced into the bloodstream. Aspiration should continue for 5 to 10 seconds, particularly with narrow gauge needles. If blood or any other fluid is obtained, the needle is withdrawn and another injection site chosen.

Ninety-seven of 101 nurses reported always performing this maneuver, only 4 not doing so; and 94 remembered being taught to do so, 7 replying that they had not been so taught. It would seem that the importance of this maneuver is well known and properly taught, perhaps because the reason for its performance is more self-evident than are those of other details of the

technique of intramuscular injection. Of the nursing texts examined, more made reference to this maneuver than to any other specific detail of the technique of intramuscular injection.

INJECTION (Fig. 1-6). A slow rate of injection allows time for distention of an accommodating space within the muscle, sparing the patient the pain sensations of the pressure-sensitive nerves within the muscular tissue. In my observation, more frequent even than a hasty rate of injection in causing unnecessary pain is the failure to have the patient "toe in" to relax the gluteal muscle. A contracted muscle cannot receive the intramuscular injection without marked increase in pressure, producing both pressure pain within the muscle and painful subcutaneous leakage outside the muscle.

A bubble of air in the syringe barrel held upright provides the means for clearing the needle of medication before withdrawal, thus reducing the dripping trail of medication through the subcutaneous tissues on withdrawing the needle.

MASSAGE (Fig. 1-7). Deep, firm massage of the muscle tissue favors spread of the medication through a wider area of tissue, increasing the area of absorption and decreasing the intensity of discomfort. Massage should be performed with the muscle relaxed by the "toed in" position, for massage of a contracted muscle may increase subcutaneous leakage. The importance of massage in relation to diminishing pain or discomfort will vary with the quality of medication used; but for the purpose of providing a wider surface for absorption massage is an important part of all intramuscular injections. Even slowly-absorbed depot material requires an absorbing surface, all the more so to prevent pocketing and walling-off by tissue reaction.

Seventy-two nurses reported always using massage after intramuscular injection, while 29 stated they did not always use it. One of the latter wrote that it "depends on the medication." In reply to the question whether they had been originally taught to massage the injection site, 81 wrote "yes," 5 "sometimes," 14 "no," and 1 did not answer. Of the 5 who wrote "sometimes," 4 added that it depends on the medication."

The Anterior Gluteal Injection Site. A simpler and safer method of intramuscular injection has become available with the introduction in 1954 by von Hochstetter[2], at the Anatomical Institute of the University of Fribourg in Switzerland, of a new area of choice for intramuscular injection, the anterior gluteal region. In 1955, von Hochstetter published a detailed review[3] of the extensive literature which has accumulated on the damaging effects of posterior gluteal injections, and analyzed their causes. The anatomy of the posterior gluteal and of the new anterior gluteal injection sites was detailed in a subsequent paper[4], in which an extensive favorable experience with the new injection site was reported. Figures 4 and 5, adapted from von Hochstetter, indicate the anatomical localization of the anterior gluteal injection as seen in frontal and obliquely horizontal body sections.

The anterior gluteal region actually provides a greater thickness of gluteal muscle into which to inject, consisting of the gluteus medius and minimus muscles, rather than the gluteus maximus muscle found posteriorly. No significant penetrating nerves or blood-vessels are found in this area, which is served by multiple small nerve and blood vessel branches. At its depth, it is sealed off by bone from underlying vital structures. The direction of the muscle bundles is such as to prevent a run-off of injected material toward

the sciatic nerve, in contrast to the gluteus maximus muscle bundles of the posterior injection site. The layer of subcutaneous fat in the anterior gluteal region is always thinner than that over readily localized by palpation of available bony markings by simple maneuvers. It can be used in any body position, and is especially suitable for use in patients lying on their backs.

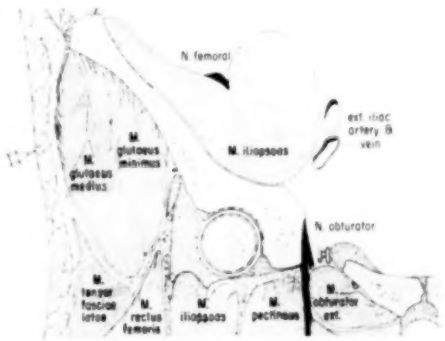

Fig. 4.—Frontal section through right half of pelvis at site of ventral gluteal intramuscular injection. Position of needle is indicated. (Adapted from von Hochstetter[4].)

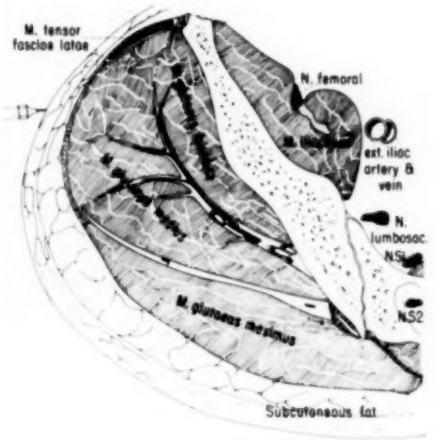

Fig. 5.—Cross section through left half of pelvis, from anterior superior iliac spine to posterior inferior iliac spine. Position of needle at ventral gluteal injection site is indicated. (Adapted from von Hochstetter[4].)

the posterior buttock, thus reducing the hazard of subcutaneous deposit of medication by short needles. Furthermore, the subcutaneous tissue is looser here, allowing its thickness easily to be determined by raising a skin fold. It is a cleaner area, freer of fecal organisms on the skin surface. It can be

Schmidt[6], at the University of Basel in Switzerland, in a comparative study found the new ventral gluteal injection site preferable to the traditional posterior gluteal site. Despite accurate technique, posterior injections with dye into cadavers resulted in some subcutaneous deposits, while none followed

anterior gluteal injections. In deliberate attempts to misdirect injected dye, using an 8 cm. long needle, he found direct damage of greater vessels or nerves to be impossible from the anterior injection site, though lesser nerve branches could be reached. Comparing 500 injections of each of the two types in both ambulatory and hospitalized patients, he found that the intensity and duration of pain induced was approximately the same for the two methods.

fingertip finds the crest of the ilium in order to slide just below it and rest there. As the palm of the hand then rests on the lateral aspect of the anterior pelvic region, the site of injection is unmistakably outlined by the confines of the triangle formed by the V of the two fingers and the horizontal ridge of the iliac crest above (Fig. 6). A right handed nurse injecting into the patient's left side will reverse the positions of the two fingers of the left hand, placing the third finger on the

Fig. 6.—The two-fingered technique of localization of injection site for ventral gluteal intramuscular injection.

The anterior gluteal injection site is readily localized by palpation (von Rechenberg and Schmidt[5]), as shown in Fig 6. The anterior superior iliac spine (actually a bossy prominence at the junction of the iliac crest with the medial vertical border of the iliac bone) is identified, and the tip of the index finger rested on it. The adjacent third finger is widely abducted (to form a V with the index finger as in the Swiss oath or in Sir Winston Churchill's V-for-victory sign), and its anterior superior iliac spine and the index finger just below the iliac crest laterally. In very obese patients, it may be necessary to lift the abdominal panniculus upward and medially away from the injection site; this is easily done by the patient or an assistant.

The needle is thrust through skin and muscle in a single movement, pointing slightly toward the head, to a depth of 3 to 4 cm. (one to 1½ inches) and the medication injected slowly, after first aspirating for the rare possibility of

having entered a blood vessel. If the needle should be pointed too far cephalad, it is apt to encounter bone, in which event the needle is withdrawn partially before injecting. The technique is simple and easily learned.

The method of anterior gluteal intramuscular injection was introduced into the wards of the Medical Service at this hospital in May, 1959, and has been used routinely since then. Having learned the simple technique, nurses quickly became enthusiastic over its advantages in not having to turn patients and in the marked reduction in the number of details of technique required by the posterior gluteal injection. With increased experience, they became accustomed to the absence of sequelae in the form of painful subcutaneous indurations, patients' discomfort in lying on their injected buttocks, and tingling nerve injuries involving the legs. Although an energetic program of teaching and encouragement was required to establish the new technique on the wards of the Medical Service, its spread through the remainder of this large hospital was initiated entirely by the Nursing Service as a result of the enthusiasm of the nurses of the Medical Service.

In nearly 2 years of experience with this method, we have encountered no morbidity. Pain, as Schmidt found, is about the same as in posterior gluteal injections, though painful sequelae are not seen. Patients who are ambulatory sometimes complain of greater pain on walking than they had experienced after posterior gluteal injections. On the other hand, bedfast patients have had none of the pain previously complained of when lying on their injected buttocks. Our experience is in agreement with the Swiss authors that this technique of intramuscular injection, if not ideal, is the best available.

Summary. 1. The accepted traditional technique of posterior gluteal intramuscular injection is reviewed. The necessity for meticulous care in carrying out the details of that technique is emphasized, if needless pain and morbidity are to be avoided.

2. A questionnaire of nursing practice disclosed remarkable deviation from the details of accurate technique, and revealed a high incidence of needless infliction of pain. Two examples of sciatic neuritis of the author's recent personal experience are cited.

3. A new method of intramuscular injection, proposed by Swiss authors, utilizes the ventral gluteal musculature and is free from hazard of injury to large vessels or nerve trunks. It has proved to be free of morbidity, simple to teach and practice, and almost foolproof of error. It deserves wide adoption.

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SUMMARIO IN INTERLINGUA

Notas in Re Technicas de Injection Intramuscular

1. Es revistate le acceptate technica traditional del injection intramuscular postero-gluteal. Le necessitate de observar meticulosemente le detalios de ille technica es sublineate. Alteremente evitabile dolores e morbiditate pote resultar.

2. Un questionario in re le practica de infirmeras hospitalari revelava un grado remarcabile de deviation ab le detalios del accurate technica, reflectente un alte incidentia de non-necessari infliction de dolor. Duo exemplos de neuritis sciatic es citate ab le experientia personal del autor.

3. Un nove methodo de injection intramuscular, proponite per autores switze, utilisa le ventral musculatura gluteal e es libere del risco de injuria a grande vasos o a truncos de nervo. Illo se ha provate libere de morbiditate e es simple a inseniar e a practicar. Errores es quasi impossibile. Le methodo merita esser adoptate extensemente.

USE OF ARGININE HYDROCHLORIDE AS AN ACIDIFYING AGENT

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Potentiation of diuretic action of various drugs, particularly the mercurials, following the induction of a hyperchloremic acidosis has been recognized for many years (Pitts and Sartorius[16], Gamble, Blackfan and Hamilton[6], Axelrod and Pitts[1], Hilton[8], Sartorius, Roemmelt and Pitts[19]). Ammonium chloride (Hilton[5]), calcium chloride (Hollister *et al.*[9]), hydrochloric acid, and, more recently, lysine hydrochloride (Ruben *et al.*[18], Lasser, Schoenfeld and Friedberg[11]) have been utilized to produce such a metabolic state. Ammonium chloride has probably been the most widely used agent, but its use is inadvisable in patients with liver disease, since it may induce the syndrome of hepatocerebral intoxication (Ticktin, Fazekas and Evans[21], Manning and Delp[14], Calkins and Delp[3]).

Recently, Britton[2] and Gidekel *et al.*[7] have reported the effects of the intravenous administration of arginine hydrochloride on acid base balance. This material would appear to obviate the dangers of administration of ammonium precursors in the patient with liver disease and may actually contribute to lowering blood ammonia levels in such patients via stimulation of urea synthesis (Manning[13]), or induction of an acidosis which may also alter ammonia toxicity by changing the ammonium ion/ammonia ratio.

This study was undertaken to determine the effectiveness of the oral administration of arginine hydrochloride as an acidifying agent, in patients in whom ammonium salts or ammoniagenic materials might be toxic.

Materials and Methods. Thirty-one patients were studied, 18 of whom had primary hepatic disease, 9 had congestive heart failure of varied etiology, 3 had neoplastic disease and one patient had chronic glomerulonephritis. They all had ascites or peripheral edema, or both, were hospitalized, and the majority were on 1650 mg. sodium diets. The age range was 3 to 76 years with 2 being under 20 years of age. Eighteen of these received at least 30 gm. in one 48-hour period.

Arginine hydrochloride* was administered in 10-gm. amounts in salt-free tomato juice. This quantity provides approximately 48 mEq. of hydrogen and chloride ions.

Urinary and serum electrolytes were determined with a Coleman Model B flame photometer by standard techniques. Blood pH was determined between 8:00 and 9:00 a.m. on heparinized capillary blood, obtained by finger puncture. A Cambridge research model pH meter with a Sanz capillary microelectrode was used. A Gebruder-Haake type F constant temperature water bath was used to maintain electrode temperature at 38° C. This apparatus was portable and allowed determination of blood pH's at the bedside.

Results. In the 18 patients receiving at least 30 gm. of arginine hydrochloride, during a 48-hour period, there was a drop in capillary blood pH or a rise in serum chloride, or both. The range of pH change was 0.06 to 0.41

*Kindly supplied by Central Research Laboratories, General Mills, Inc., Minneapolis, Minn.

pH unit and 6 to 40 mEq. per L. for chlorides. A graphic representation of the pre- and postadministration changes in serum pH, carbon dioxide, serum chloride and weight values are noted for this group in Fig. 1. In the other 13 patients who received smaller and less frequent amounts, the changes showed considerable variation. The following cases are illustrative of the changes induced.

in any significant diuresis. Arginine hydrochloride was administered at the dosage level shown and a rise in serum chloride, drop in CO_2 and decrease in pH are noted. During periods of arginine hydrochloride administration there was an increase in chloride excretion and a mild weight loss, amounting to approximately 5 pounds. Two milliliters of meralluride and 0.5 gm. of aminophyllin were administered at the intervals shown, with a subsequent marked diuresis, weight loss and increased sodium and chloride excretion. During these periods of diuresis, there was no

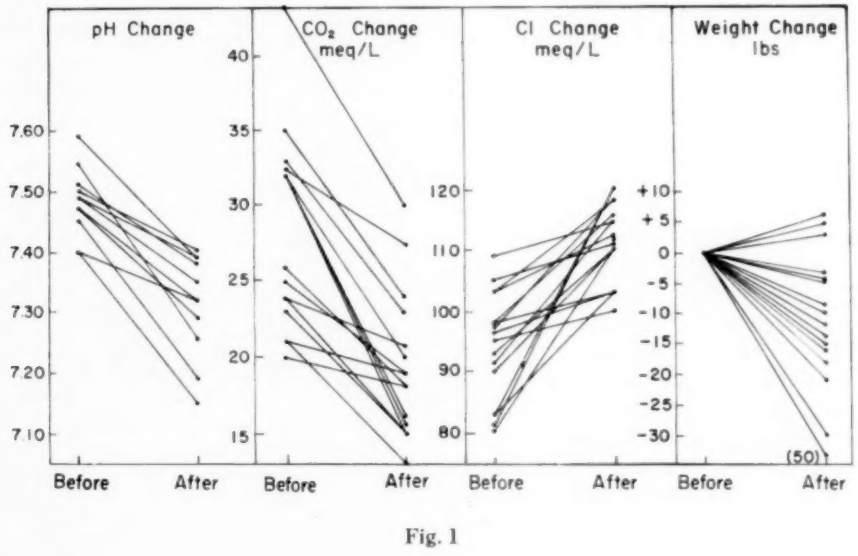

Fig. 1

Case Reports. CASE 1. M.C., a 42-year-old Negro woman, was admitted to the University of Kansas Medical Center on four separate occasions between May, 1958, and August, 1959. On each admission, the patient had massive ascites and peripheral edema. The clinical diagnosis of post-necrotic cirrhosis of the liver was subsequently confirmed at necropsy. The clinical course of the patient's third hospital admission is summarized in Fig. 2. Prior to the administration of arginine hydrochloride, she had become refractory to mercurial diuretics and to chlorothiazide compounds. At the time of this admission, she showed evidence of a hypokalemic, hypochloremic alkalosis with a blood pH of 7.60, chlorides of 91 and potassium of 2.7 mEq. per liter. The administration of 1 ml. Mercuhydrin on the 5th hospital day did not result evidence of hepatocerebral intoxication. The patient had previously had three episodes of hepatic coma and succumbed on her fourth and final visit in hepatic coma incident to bleeding from esophageal varices.

Arginine hydrochloride by mouth induced in this patient a hyperchloremic acidosis without apparent toxic effect, and as such, enhanced the diuretic response to mercurial administration.

CASE 2. G.D. was a 68-year-old woman with arteriosclerotic heart disease, pyelonephritis and congestive heart failure. Her first admission is summarized in Fig. 3. Intermittent administration of arginine hydrochloride, followed by mercurial injections, produced the diuretic effect as shown. pH's were not determined in this patient. She lost 50 pounds of body weight, her congestive failure

cleared and she was discharged. No toxic effects were observed, except some mild diarrhea toward the end of each period of arginine administration. Her second hospitalization is shown in Fig. 4. Again during the administration of arginine hydrochloride she showed an increase in sodium and chloride excretion and weight loss, when challenged with mercurial diuretics.

CASE 3. W.W. was a 34-year-old man with classic findings of Laennec's cirrhosis with massive ascites accumulation, edema and severe jaundice. The patient had been treated in his local hospital with chlorothiazide, mercurial diuretics and had become refractory to these measures. Following administration of arginine hydrochloride, he showed a mild weight loss and again showed responsiveness to mercurial diuretics as noted in Fig. 5. His edema and ascites cleared considerably, but he developed a profound hyperchloremic acidosis, and, following rupture of a perirectal abscess, lapsed into hepatic coma and died.

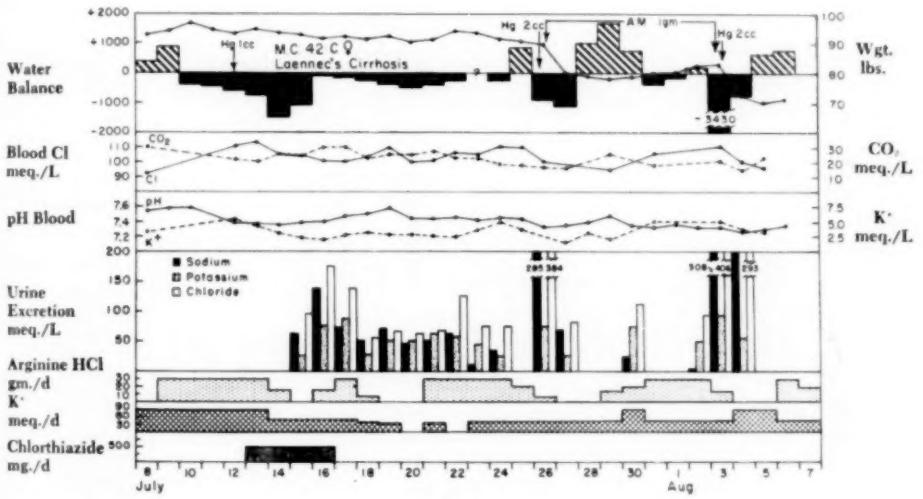

Fig. 2

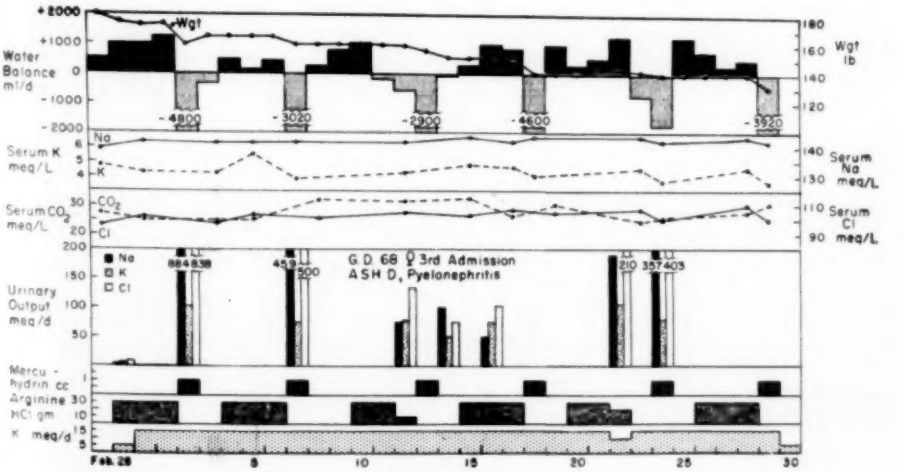

Fig. 3

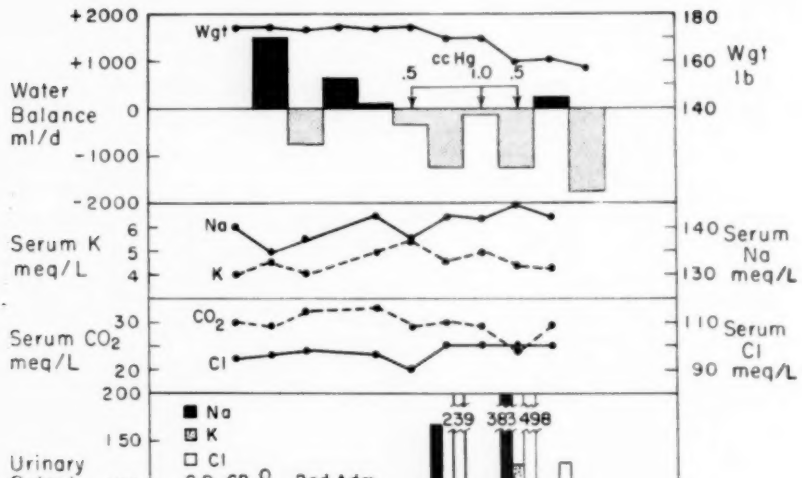
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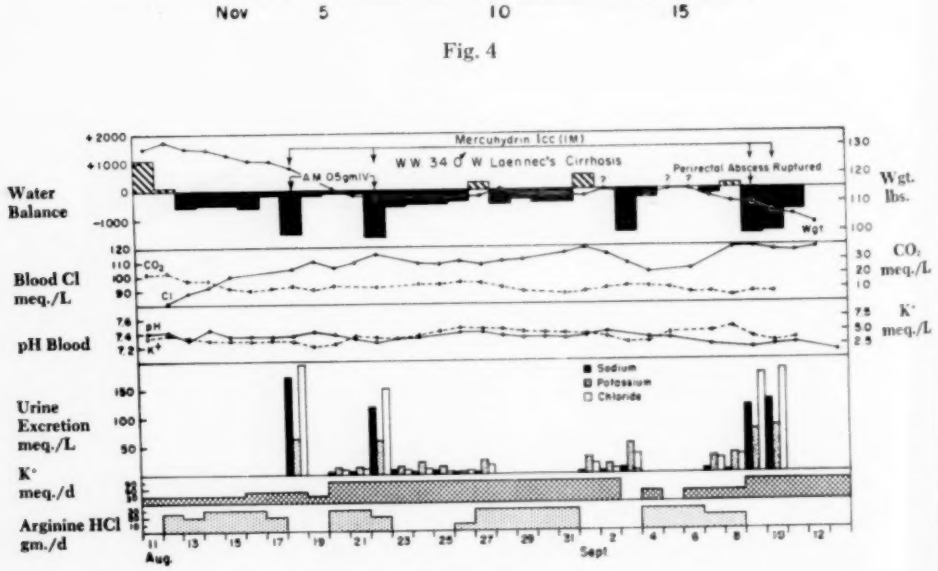
Fig. 4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Chloride
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Fig. 5

It would appear that in this patient, the administration of arginine hydrochloride was an assisting factor in returning his responsiveness to mercurial diuretics; however, the continued administration of this agent probably led to a profound irreversible hyperchloremic acidosis. For this reason, it is suggested that arginine be given on an interrupted schedule with frequent observation of serum chloride and CO_2 values and blood pH, if available.

Discussion. It would seem apparent that the oral administration of arginine hydrochloride is an effective means of producing a hyperchloremic acidosis. This may be accomplished without significant side effects exclusive of some diarrhea, if precautions are taken to avoid a severe acidotic state. Given in the fashion outlined, it seems to return the responsiveness to mercurial administration, a phenomenon which has been previously observed with other acidifying agents.

No episodes of hepatocerebral intoxication could be attributed to the administration of arginine hydrochloride. It would seem, therefore, to be a suitable agent in patients susceptible to ammonia intoxication, when the production of an acidotic state is desired. In addition to the observation that it may augment the diuretic responsiveness, the production of an acidosis may be beneficial in patients with hepatic decompensation, by altering the physical-chemical equilibrium of $NH_3 \rightleftarrows NH_4^+$ (Stabenau, Arren and Rall[20], Jacquez[10]). In addition, it may promote the renal excretion of ammonia (Miln, Scribner and Crawford[15], Madison and Seldon[12], Davies and Yudkin[5], Rector, Seldon and Roberts[17], Davies[4], Tyor *et al.*[23]) and stimulate the incorporation of ammonia into urea by accelerating the hepatic synthesis of urea (Manning[13]).

Summary. 1) Thirty-one patients were given arginine hydrochloride by mouth and all patients, receiving 30 gm. or more, within 48 hours showed a drop in pH and rise in serum chloride. The pH drop ranged between .06 to .41 pH unit and the chloride rise from 6 to 40 mEq. per L.

2) The induction of a hyperchloremic acidosis was associated with an augmented response to the administration of organic mercurial diuretics.

3) No patient developed hepatocerebral intoxication which could be directly attributed to the administration of the arginine hydrochloride.

4) One patient developed a profound hyperchloremic acidosis which was irreversible and may have significantly contributed to his demise. No other toxic effects, except occasional diarrhea which responded to reduction of dosage, were noted.

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SUMMARIO IN INTERLINGUA

Le Uso de Hydrochloruro de Arginina Como Agente Acidificatori

1. Trenta-un patientes recipeva hydrochloruro de arginina per via oral. Omnes qui recipeva 30 g o plus intra 48 horas manifestava un reduction del pH e un augmento del chloruro seral. Le reduction in pH variava inter 0,06 e 0,41 unitates de pH. Le augmento del chloruro variava inter 6 e 40 mEq per L.

2. Le induction de un acidosis hyperchloremic esseva associate con un augmento del responsa al administration de organic diureticos mercurial.

3. Nulle del patientes disveloppava un intoxication hepato-cerebral que poteva esser attribuite directemente al administration de hydrochloruro de arginina.

4. Un patiente disveloppava un profunde acidosis hyperchloremic que esseva irreversibile e que ha possibilemente contribuite significativemente a su decesso. Nulle altere effectos toxic esseva notate, excepte un diarrhea occasional que respondeva al reduction del dosage.

AIR POLLUTION AND DAILY MORTALITY*

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The rapid population and industrial growth of cities, especially since World War II, has resulted in a steady increase in the amount of pollutants emitted into the atmosphere. Already in many areas the natural capacity for the disposal of airborne wastes has been reached. It has been estimated for the United States as a whole that the damage to live-stock, vegetation and materials alone exceeds three or four billion dollars yearly (Gustavson[14], 1958). The deleterious effects on human health and well-being are more difficult to quantify, though evidence from a variety of epidemiologic studies suggests a parallelism between exposures to polluted air and illness.

Some of the population studies which suggest air pollution as a possible menace to health can be summarized as follows:

1. The areas with the heaviest air pollution load tend to rank high in death rates for a number of diseases (Daly[7], 1959; Fairbairn and Reid[11], 1958; Pemberton and Goldberg[26], 1954; Manos and Fisher[22], 1959).

2. There exists a large difference between urban and rural areas for specific causes of death such as coronary heart disease and various forms of cancer (Lilienfeld[20], 1960; Enterline *et al.*[10], 1960; Levin *et al.*[18], 1960; Clemmesen and Nielsen[6], 1957).

3. British immigrants have a higher rate of lung cancer than the native population of British stock in New Zealand (Eastcott[9], 1956) and in South Africa (Dean[8], 1959).

In addition to these studies, which deal with long-term or chronic effects, there is evidence that pollution in unusually high concentrations, under certain topographical and meteorological conditions, can cause immediate or acute damage to exposed human populations. This hazard has been demonstrated by the catastrophic episodes which occurred in Donora (Schrenk *et al*[29], 1949), in the Meuse Valley (Firket[12], 1931), and in London, 1952 (Ministry of Health Report[25], 1954; Logan[21], 1956). In these disasters there was an immediate and pronounced increase in the incidence of morbidity and mortality, particularly from cardiac and respiratory causes.

These severe episodes have directed attention to possible acute effects from exposure to lesser concentrations of air pollutants. Attempts to assess this hazard have been made, using daily records of London (Boyd[2], 1960; Martin and Bradley[23], 1960) and Los Angeles County (Mills[24], 1957; Breslow and Goldsmith[3], 1958). In these studies various indices of the daily air pollution level were correlated with the corresponding mortality pattern. The results, taken as a whole, are ambiguous and difficult to interpret. For ex-

*This study was supported in part by a contract with the Air Pollution Division of the United States Public Health Service.

ample, Mills[24] (1957), concluded that smog caused an appreciable number of deaths in Los Angeles County, but Breslow and Goldsmith[3] (1958), using essentially the same observations, failed to confirm these conclusions. It should be noted that these studies were directed at ascertaining not the long-term effects but the immediate terminal response of man to variations in air pollution.

Mortality is, at best, a rough indicator of an acute response. Death is a terminal event which follows a long sequence of circumstances; its occurrence at a particular time is attributable, in large part, to the person's general disease history. As Carey *et al.*[4] (1958) put it, man's health depends upon the resultant of two opposing forces, the duration and intensity of the exposure or insult and the power of the defensive mechanism of the body to oppose or repair that injury.

The lack of an easily measurable and sensitive biological indicator is a serious limitation to ascertaining the effects from low level air pollution. Mortality is commonly used as an indicator because it is easily measurable but it is far from sensitive.

This paper reports some further analyses of the environment and mortality data of Los Angeles County and indicates some of the statistical difficulties encountered in correlation studies.

PROBLEM OF TIME SERIES. Measurements observed in a temporal sequence are generally referred to as time series. The main difficulty in the analysis of time series is that the successive observations are not statistically independent, and hence cannot be treated as if they were the result of random sampling. Autocorrelation is the term used to describe the tendency of successive observations to be interdependent. This interdependence can arise from the presence of regular and irregular oscillations which have been generated by different causal systems. If autocorrelation exists, the calculated correlation coefficients between the variables are suspect and should be interpreted with great caution (Yule[30], 1926; Quenouille[27], 1952).

As an illustration of the time dependency of the variables under consideration, Fig. 1 gives for each month the oxidant concentration (pphm), carbon monoxide concentration (ppm), temperature in degrees Fahrenheit, and death rate (per 100,000) for cardiac and respiratory causes in Los Angeles County, 1956 to 1958. The plotted values for the two indices of air pollution were obtained by averaging the daily maxima of five monitoring stations. The temperature is the monthly average of the daily maxima in downtown Los Angeles. All of the variables exhibit a recurrent cyclic pattern, that is 12 months in duration plus some irregular oscillations. It should be noted, too, that the oxidant level and temperature are roughly 6 months out of phase with the deaths. The cyclic patterns of the oxidant and carbon monoxide concentration are in agreement with those observed by Hamming, MacPhee and Taylor[15] (1960).

SEASONAL PHENOMENA. Seasonal influence of weather on morbidity and mortality is an age-old problem. It was explicitly mentioned in the writings of Hippocrates and has been the subject of many studies over the years. Recent studies are those of Hisdal[16] (1953), Reed[28] (1924), Kosambi and Raghavachari[17] (1954), Lewis-Faning[19] (1940), and Chang[5] (1952). Other references are given in the review paper by Gordon and Erhardt[13] (1958).

To give some indication of the influence of weather on mortality, smoothed curves drawn to the average monthly death rates (per million) from circula-

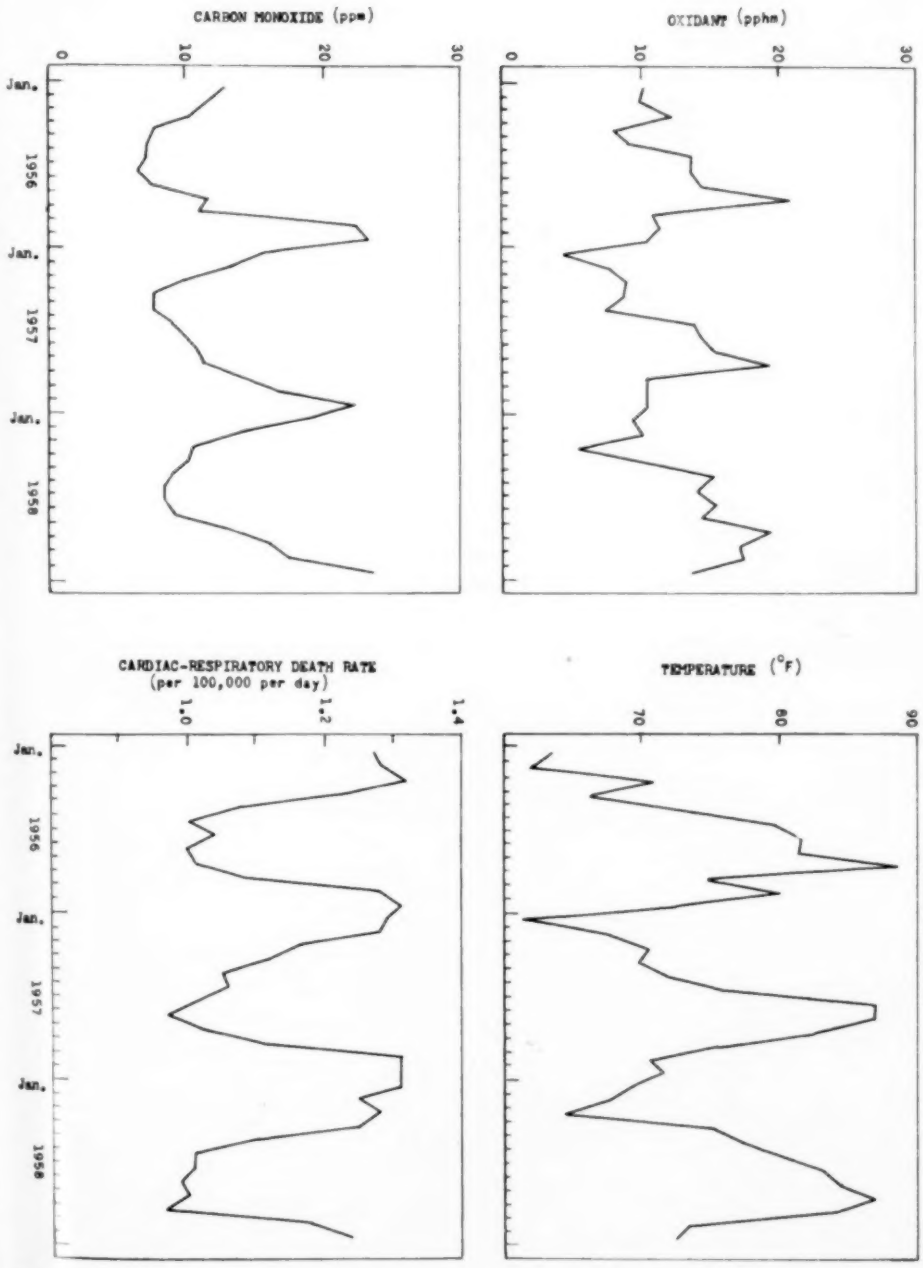

Fig. 1.—Maximum oxidant, carbon monoxide, temperature and death rate for cardiac and respiratory causes, Los Angeles County, 1956–1958.

tory diseases, bronchitis and other respiratory diseases in England and Wales and in Australia, over a few widely spaced years are shown in Fig. 2. These countries were selected not only because they publish excellent summaries of their vital statistics, but also because they offer an interesting contrast in geographic location and industrial complex. From the figure it is seen that deaths due to circulatory and respiratory causes occur much more fre-

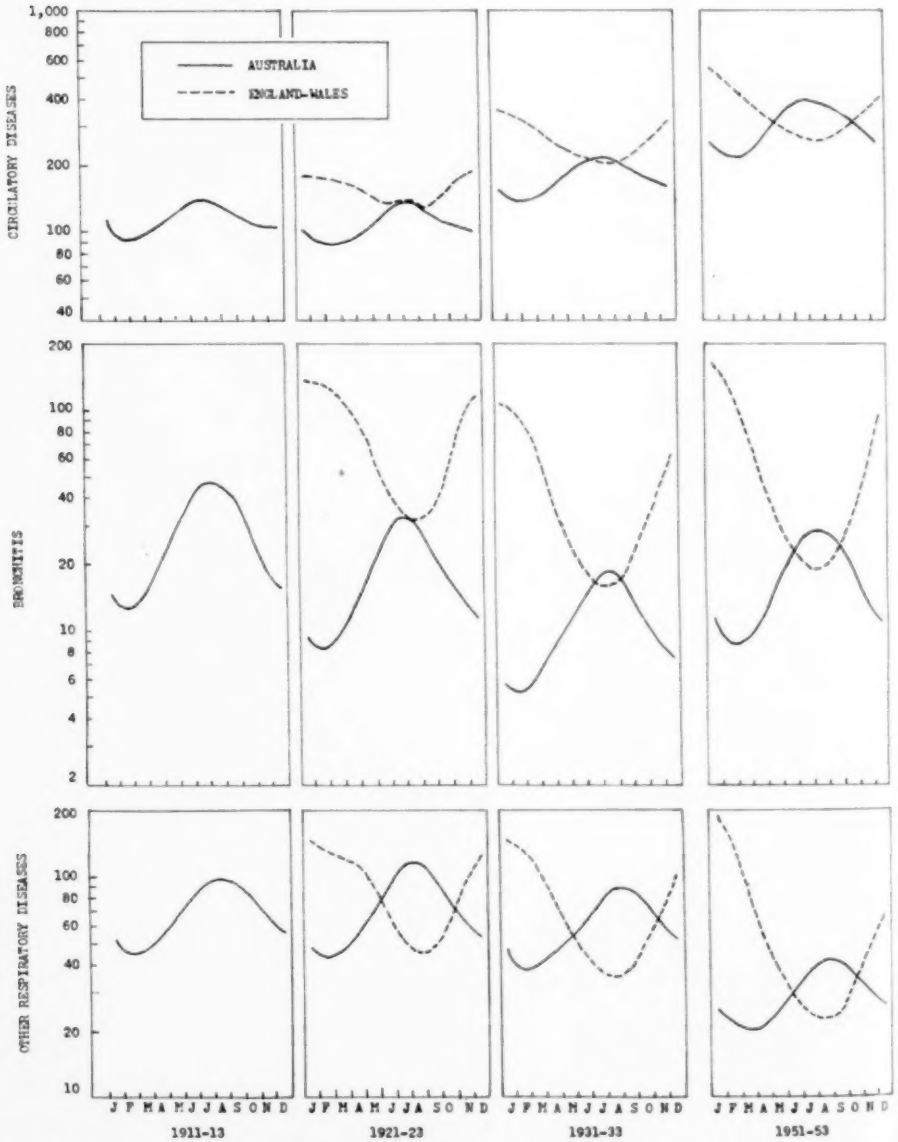

Fig. 2.—Average monthly death rate for bronchitis, other respiratory and circulatory diseases Australia and England-Wales (per 1,000,000)

quently in both countries in their winter season. The consistent seasonal pattern over the decades clearly indicates a strong relationship between mortality and climate.

REMOVAL OF THE SEASONAL COMPONENT. In time series analyses, it is customary to isolate and remove the periodic or seasonal component from the variables and to base conclusions on the residual deviations from the seasonal cycle. That is, the seasonal pattern is considered a cyclic fluctuation imposed on the series by external forces. A common method for analyzing the seasonal component of a trend-free time series, when the length of the cycle is determined independently of the response, is harmonic or Fourier analysis (Bliss[1], 1958). This consists of fitting to observations, such as shown in Fig. 1, a finite series of sine and cosine functions. This method was used for the data from Fig. 1 and the fitted curves are shown in Fig. 3. For mortality, oxidant, and temperature the first harmonic, a simple sine curve, was found adequate for describing the time relationship; for carbon monoxide the first three terms of the Fourier series were needed. Elimination of the seasonal component was accomplished by expressing each daily observation as a deviation from the fitted curve.*

CORRELATION BETWEEN TIME SERIES. Elimination of the seasonal component does not necessarily mean that the remainder of the time series is free of autocorrelation. Other systematic oscillations exhibiting no obvious regularity could be operating. To measure the extent of these non-random fluctuations, autocorrelation coefficients were computed and are shown in Table 1. The first row gives the correlation between successive observations, the second row between observations 2 days apart, and the third row gives the partial correlation coefficient for observations 2 days apart when the effect of the intermediate day has been eliminated. The relatively high value for r_{12} for environmental variables indicates that the successive daily residuals still tend to be correlated, and the low values for $r_{13 \cdot 2}$ reveal that the correlation does not extend beyond the adjacent day.

TABLE 1.—AUTOCORRELATION COEFFICIENTS OF RESIDUALS LOS ANGELES COUNTY, 1956–1958

		Oxidant	*Temperature*	*Carbon Monoxide*	*Cardiac and Resp. Deaths*
Correlation Between Successive Daily Observations	r_{12}	.56	.71	.54	.14
Correlation Between Observations Two Days Apart	r_{13}	.24	.42	.18	.14
Partial Correlation Coefficient	$r_{13 \cdot 2}$	−.11	−.16	−.15	.12

Correlation or association between two variables is said to exist if changes in one are accompanied systematically by changes in the other. When the variables are studied over time, the most meaningful association is not necessarily obtained by correlating the simultaneous values of the variables; there could be a delay in the influence of one variable on the other. To ex-

*For illustrative purposes, curves based on the combined data for the 3 years are shown in Fig. 3. For the analysis, curves for each individual year were fitted and the residual refers to the difference between the daily observation and the fitted curve for that year.

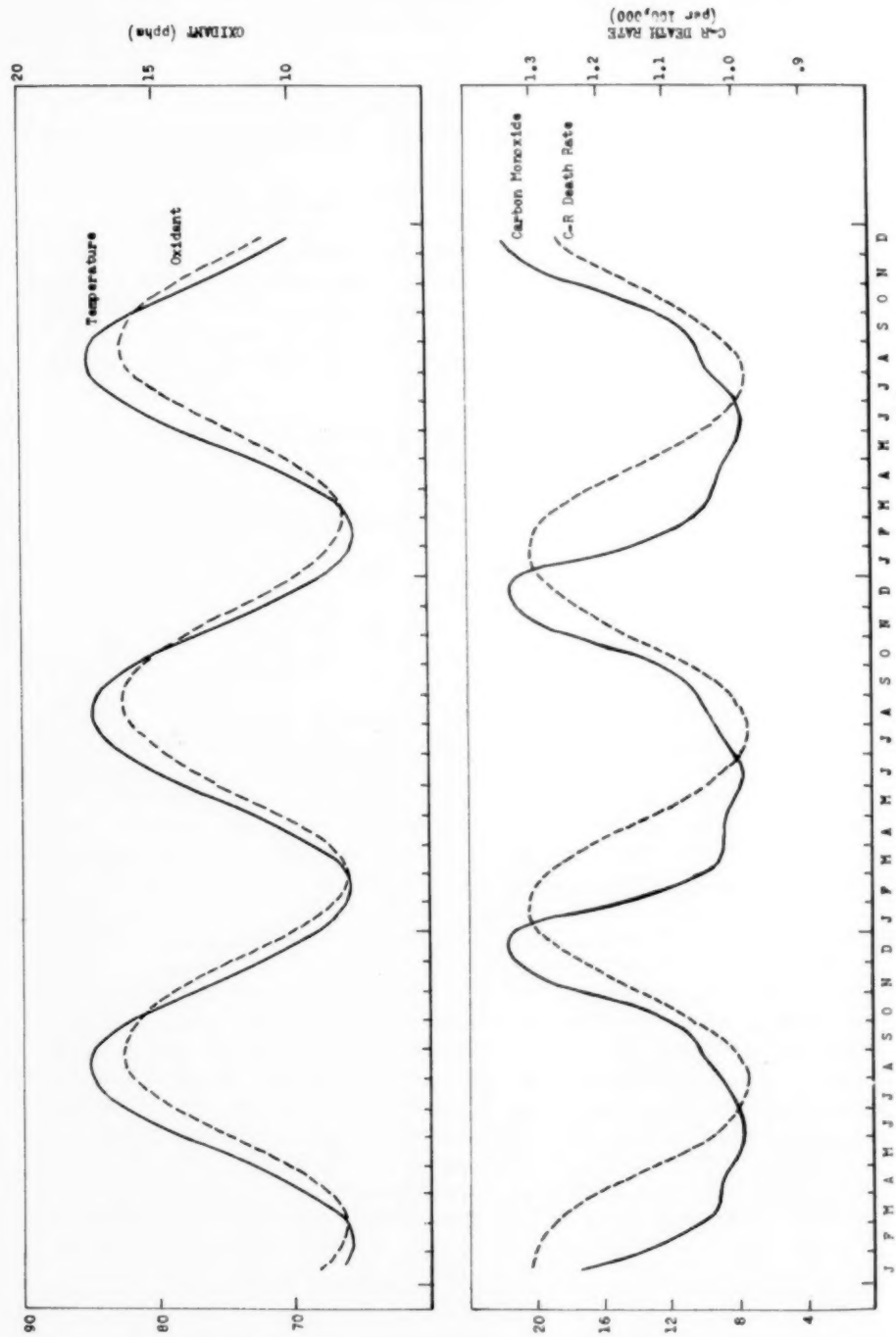
OXIDANT (ppha)
C-R DEATH RATES (per 100,000)
Temperature
Oxidant
Carbon Monoxide
C-R Death Rate
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1956
1957
1958
TEMPERATURE (°F)
CARBON MONOXIDE (ppm)

Fig. 3.—Fitted Fourier curves to data from Fig. 1.

amine this possibility, coefficients for the correlation of atmospheric variables with deaths, 0, 1, 2, 3, and 4 days later were computed. These correlations are referred to as lag-cross correlation coefficients and are shown in Table 2.

By combining the information in Tables 1 and 2, the association between the variables can be evaluated by a method of Quenouille[27] (1952). For example, the correlation between carbon monoxide and deaths the following day would be given by the lagged partial correlation coefficient:

$$r_{C_i D_{i+1} \cdot C_{i-1} D_i} = 0.04$$

This is interpreted as the correlation between C and D one day later when the effects of one-day autocorrelation in both variables have been eliminated. To test the significance of this value the ordinary statistical test for partial correlation coefficients can be applied. In view of the very low values of the correlations shown in Table 2, none of the lagged partial correlation coefficients for the various combinations is statistically significant.

TABLE 2.—LAG CROSS CORRELATION COEFFICIENTS OF RESIDUALS LOS ANGELES COUNTY, 1956–1958

		Cardiac and Respiratory Deaths				
		D_i	D_{i+1}	D_{i+2}	D_{i+3}	D_{i+4}
Oxidant	O_i	.10	.10	.03	−.05	−.05
	O_{i+1}	.09				
Temperature	T_i	.16	.08	.04	−.04	−.08
	T_{i+1}	.15				
Carbon Monoxide	C_i	.14	.08	.06	.05	.01
	C_{i+1}	.08				

Summary. Analysis has been made of the relationship between various environmental factors and daily cardiac and respiratory deaths in Los Angeles County from 1956 to 1958.

It was found that the variables, when studied over time, exhibited a distinct seasonal pattern plus some irregular oscillations. The seasonal component from each series was isolated and removed by the techniques of harmonic analysis.

A correlation analysis, adjusting for the autocorrelation remaining in the residuals, indicated that changes in the daily air pollution level, as measured by the oxidant and carbon monoxide concentration, exerted no detectable influence upon the day-to-day mortality pattern.

It is emphasized that these findings, which deal only with the acute terminal response of man to variations in the daily air pollution level, do not mitigate the importance of air pollution as a possible menace to the healtl. and well-being of man.

ACKNOWLEDGMENTS: The authors are grateful to Mr. Norman Perkins for tabulating assistance, to Mr. Lynn Hayward for providing a computer program for the harmonic analysis, to Mr. N. S. D'Andrea Dubois, Jr., for performing the analysis on the IBM 704 Digital Computer, and to Dr. Carl Hopkins for his many helpful suggestions.

Fig. 3.—Fitted Fourier curves to data from Fig. 1.

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SUMMARIO IN INTERLINGUA

Pollution del Aere e Mortalitate Diurne

Esseva analysate le relation inter varie factores ambiental e le diurne mortalitate cardiac e respiratori in le Contato Los Angeles ab 1956 ad 1958.

Esseva trovate que le variabiles, quando studiate in relation al tempore, exhibiva un distincte rhythmo saisonal e etiam varie oscillationes irregular. Le componente saisonal in cata un del series esseva isolate e removite per le technicas del analyse harmonic.

Un analyse correlational, que corrigeva le effecto del autocorrelation remanente in le residuos, indicava que alterationes in le nivellos diurne del pollution del aere (mesurate per le concentrationes de oxydante e de monoxydo de carbon) exerceva nulle detegibile influentia super le comportamento del cifras de mortalitate ab un die al altere.

Es sublineate le facto que iste constatationes—que concerne solmente le acute responsa terminal del homine a variationes in le diurne pollution del aere—non reduce le importantia del pollution del aere como un menacia possibile pro le salute e le ben-esser del homine.

THE VALUE OF TRIAMCINOLONE IN ALLERGIC ASTHMA: A COMPARATIVE STUDY

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WITH THE ASSISTANCE OF

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Since 1949, when Hench and Kendall[4] introduced the use of cortisone in arthritis, remarkable progress has been made with the use of these agents not only in arthritis but also in allergic diseases. The greatest disadvantages of the earlier corticoids* were the frequency and at times marked side-effects especially those resulting from the sodium and water-retaining qualities of these agents. Further research by the biochemists was sucessful in producing newer preparations which minimized these side-effects while at the same time retaining their potent pharmacologic and physiologic effects. As a result, some of the newer corticoids have practically replaced the older ones in the treatment of allergic diseases. One of the newer ones is triamcinolone (Bernstein[1]) which has been in clinical use now for several years. According to several reports in current literature (Criep[2], Feinberg, Feinberg and Fisherman[3], Sherwood and Cooke[5]), this preparation seems more advantageous than the earlier corticoids (cortisone, hydrocortisone, prednisone, and prednisolone) in that equal or even better clinical effect can be obtained from smaller dosage with fewer side-effects. Nearly all these reports were based on clinical observations. Thus, although many of the patients who were given triamcinolone had taken the older corticoids previously, judgment as to the comparative value of the former in relation to the latter was based upon clinical impression rather than on well-controlled studies.

More than a year ago the author had an opportunity to carry out studies including a double-blind one comparing the effects of triamcinolone (as Aristocort) with other corticoids, especially with prednisone. At first a small series of patients with asthma, who previously had taken other corticoids, were given triamcinolone and the effects noted. After a 6-month clinical study of these patients, it seemed to the author that triamcinolone provided better clinical effects than did any of the previously used corticoids. But since this at best was only a clinical impression, the author attempted to provide more definite data by carrying out comparative double-blind studies. The results of these studies are the basis of the present report.

Methods and Material. The material utilized in this investigaion was furnished by the Lederle Laboratories as a research grant. Three types of tablets, labelled A, B, and C were prepared in such fashion as to be

*Since the term "steroids" refers to substances manufactured by glands other than the adrenal cortex, the term "corticoid" will be used throughout this presentation to designate those steroids associated with the adrenal cortex, namely the "corticosteroids."

indistinguishable in size, shape, and color. One tablet contained 4 mg. of triamcinolone (Aristocort), another 5 mg. of prednisone, and the third contained lactose as a control or placebo. The contents of these tablets remained unknown to the author until after the final report of the results had been sent to Lederle Laboratories. In addition, we had a supply of the regular 4-mg. triamcinolone (Aristocort) tablets (shaped in rectangular fashion rather than rounded) for use in those patients who had been taking other corticoids prior to being given the unknowns. This will be referred to in our results as the "known triamcinolone."

The patients used in the comparative study included chiefly patients from the author's private practice but also a small number of patients from the outpatient allergy clinics of Temple University Center and of the Veterans Administration*. These patients all had chronic asthma of varied duration which did not satisfactorily respond to the usual forms of treatment. Most of these patients had been taking corticoids prior to the present study. Whenever possible, all medication other than the tablets being tested was excluded during the trial period.

The procedure in most patients was as follows: Most patients who had been taking a corticoid previously were first given the known triamcinolone tablet in a dosage comparable to that of the corticoid which they had been taking. After being on this drug long enough to judge its comparative effect, the patient was given tablet A, B, or C for a period of one week or longer. In all instances the tablets were given at the same hour as the known triamcinolone tablet; also the same number of tablets were used. After our initial experience with the tablets, we found it best to have the patient take each unknown tablet for at least 2 and in some cases 3 to 4 weeks before changing to the next unknown. Thus, we found that in some patients the beneficial effect which followed the use of one unknown for a period of a week would carry over into the next week. This would make it difficult to interpret the results if only one week was used as a criterion. We, therefore, used 2 weeks in patients with milder asthma, whereas in those with severer types, the patient took each tablet for 3 to 4 weeks before changing to a different unknown. The unknown tablets were not used in the same regular sequence such as A, B, and C. In fact, we used a different sequence in succeeding patients in order not to be influenced by a knowledge of the beneficial results likely to be produced by the unknowns, especially since one was a placebo and presumably would have little or no effect. We, therefore, gave the tablets to the patients in varied sequences; for example, some patients got A, B, C; others C, A, B, and the like. After each patient had been given one or more trial periods with the different unknowns, he was given the known triamcinolone for comparative purposes. But in a few patients whose asthma seemed to be worse while on one of the lettered unknowns, it became necessary to put the patient back on the known triamcinolone to control his asthma.

At each visit a record was made of the weight, blood pressure, and changes in the clinical condition as determined by history and by examination of the chest. We were particularly careful to note the presence of any side effects such as moon face, hirsutism, purpura, weight gain, bleeding, abdominal pain, and the like. These results and the evaluation of the comparative clinical effects of the drugs were summarized at the end of the trial period.

Results. Of the total number of asthmatic patients who were utilized for the initial comparative studies between known triamcinolone and the previously taken corticoid, satisfactory data could be obtained in 19 patients, and for the double-blind study in 20 patients. Eighteen of the 19 patients in the first group improved with triamcinolone and one showed no change.

DOUBLE-BLIND STUDY. In reviewing our findings and summarizing the data, we excluded those patients who had not cooperated satisfactorily and had failed to follow the explicit directions. Those patients either discontinued the tablets when they felt better or failed to take the medication continuously or had taken more tablets than was necessary when they got more frequent attacks of asthma. We found on the whole that our private patients cooperated much better than did the clinic

*The author is indebted to Dr. L. N. Ettelson, Allergist to the Veterans Administration Outpatient Clinic, Philadelphia, Pa., for the data on some of the patients used in this study.

patients. While the number of patients given in the final report is smaller than the number who were started on the lettered tablets, we felt this group gave a truer picture of the comparative results than if those patients were included who were not cooperative. Of the 40 patients who were started on the drugs initially, only 20 were considered cooperative enough to be included in the final analysis of the results. In one patient, upper abdominal pain followed administration of tablets A and B so that C could not be given.

when taking tablet C, and a like number (10) reported no change when taking the other tablets. On the whole, tablets A and B showed little difference in the number of good or bad results, but more patients seemed better on tablet B and more patients seemed worse on tablet C.

In summary, therefore, it would seem from the double-blind study that best results were obtained when patients were taking tablet B, next best from tablet A and poorest results with tablet C. It is perhaps significant that

TABLE 1.—RESULTS OF DOUBLE-BLIND STUDY

	Questionable Improvement	*Worse*	*No Change*	*Improved*
A	2	5	10	3
B	4	4	4	8
C	2	10	4	1

Two other patients had rather severe asthma and were so much improved while taking tablets A and B that we could not risk putting them on tablet C, which previously seemed ineffectual in other patients. These 3 patients therefore did not get tablet C. Included in the final comparative double-blind studies, therefore, were 17 patients who got tablets A, B, and C, and 3 other patients who got only tablets A and B. Of the total of 20 patients in this group, 18 had taken corticoids previously for varying periods of time. But since we had records not only of the latter periods but also of the clinical condition of those patients at a similar time the year before, they were good subjects for comparative studies. The results of this comparative double-blind study are summarized in Table 1. Inspection of the results revealed that more patients (8) were improved on tablet B than on A or C. Furthermore, more patients (10) seemed worse

all patients reported improvement when given the known triamcinolone after the conclusion of the double-blind study. Since most of them had taken these tablets before taking the unknowns and had shown improvement while taking them, they undoubtedly were influenced when they took them again especially if they were not feeling well at that time. Following the completion of the study and the preparation of the final report, the envelope containing the key to the unknowns was opened. It then was revealed that tablet A was prednisone, tablet B was triamcinolone (Aristocort), and tablet C the placebo or control. This confirmed our suspicions as to tablet C and also seemed to indicate the apparent clinical superiority of triamcinolone over prednisone.

Side Effects. These will be considered first in the group of 19 patients taking known triamcinolone. On the whole, side effects in this group were minimal,

probably because most of the patients were on maintenance dosage. Thus, none of the patients receiving known triamcinolone had any weight or blood pressure change. Very mild hirsutism, not previously noticed, was present in one patient. Two patients noted dyspepsia, including nausea and heartburn and poor appetite when triamcinolone first was given. These symptoms lasted only a few days and did not recur even though the triamcinolone was continued. Four patients taking known triamcinolone noted neurologic symptoms including slight vertigo in 2, a feeling of syncope in one, and irritability in one. These were not noted when the patients were taking the previous corticoids. Two patients had palpitation. One patient taking known triamcinolone noted crampy pains in the leg muscles or toes. This was not present when taking other corticoids. Three patients, while on the known triamcinolone, had purpuric or ecchymotic spots for the first time. These came out readily after the slightest trauma. Two other patients also had purpuric spots, but these had been present in milder degree while on previous corticoids.

It was not possible to compare satisfactorily or accurately the side effects of the patients taking the different lettered tablets. Thus, if a side effect appeared while the patient was taking one tablet (for example, A), it was difficult to determine whether there was a carry-over of this effect when put on another lettered tablet. We will, therefore, make no attempt to compare the side effects among the patients taking the lettered tablets. On the whole, the side effects in patients taking the lettered unknowns were minimal. They were similar, however, to those observed with known triamcinolone or with the earlier corticoids.

Summary. Studies in 39 asthmatic patients, including a double-blind study in 20, comparing triamcinolone with prednisone, confirmed an earlier clinical impression that triamcinolone produced somewhat better clinical effects than prednisone or similar corticoids. Side effects from triamcinolone in the patients taking maintenance dosage were minimal.

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SUMMARIO IN INTERLINGUA

Le Valor de Triamcinolona in Asthma Allergic: Un Studio Comparative

Studios in 39 asthmaticos, incluse un studio bis-occulte in 20, visante a comparar triamcinolona e prednisona confirmava le previe impression clinic que triamcinolona produce effectos clinic un pauco melior que prednisona o simile corticoides. Adverse effectos lateral in patientes recipiente doses de mantenentia esseva minime.

BIOPSY OF THE PARIETAL PLEURA*

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The problem of establishing the etiology of pleurisy with effusion has long plagued the physician. For example, in three recent large series of patients with pleural effusions, the etiology of the effusion could not be determined in 17 to 40% of the patients (Engelhardt and Wilson[9], Leuallen and Carr[16], Tinney and Olsen[31]). It has become general policy to accept *Mycobacterium tuberculosis* as the etiologic agent in such patients, unless they are tuberculin negative (Roper and Waring[24]). However, it has been repeatedly demonstrated that a significant number of younger patients with presumed tuberculous pleuritis have no evidence of tuberculosis at thoractomy (Breckler *et al.*[3,4], Donohoe, Katz and Matthews[6,7], Douglass, Carr and Bernatz[8], Hill, Hensler and Brackler[14], Mitchell *et al.*[21], Richert *et al.*[22], Smith, Parsons and Daniels[27], Stead, Eichenholz and Stauss[28], Thomas and Anderson[30]), while there is an increasing frequency of primary tuberculous pleuritis in patients over 40 years of age (Abeles and Chaves[1], Bloch[2], Leuallen and Carr[16], Robertson[23]). Thus, the necessity of establishing an etiologic diagnosis in idiopathic pleural effusions becomes apparent.

During the last decade, several reports have appeared in the literature concerning the value of pleural biopsies in the diagnosis of pleural effusions. Biopsies were initially performed during thoracoscopy (Lloyd[17]) or by thoracotomy (Breckler *et al.*[3], Douglass, Carr and Bernatz[8], Small and Landman[26], Stead, Eichenholz and Stauss[28], Sutliff, Hughes and Rice[29]). Following the report of DeFrancis, Klosk and Albano[5] on Vim-Silverman needle biopsy of the parietal pleura, several investigators have reported favorable results with this technique (Donohoe, Katz and Matthews[6,7], Green[11], Heller, Kellow and Chomet[12,13], Leggat[15], Mestitz, Purves and Pollard[19], Misra and Sharma[20], Richert *et al.*[22], Samuels, Old and Howe[25], Weiss[32], Welsh[33]). This paper reports the results of needle biopsy of the parietal pleura in 42 patients and surgical biopsy of the pleura in 13 patients, all of whom were admitted to a tuberculosis sanatorium. A review of the English literature on pleural biopsies is also summarized.

Clinical Material. The records of all patients who had attempts at diagnostic pleural biopsies from January, 1955, to June, 1960, at Firland Sanatorium were reviewed. Prior to

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1955, pleural biopsies had not been used as a diagnostic technique. A total of 50 Vim-Silverman needle aspirations were undertaken in the 42 patients. Seven of the 13 patients who had surgical biopsies had had prior needle biopsies. The ages of the 48 patients in both groups ranged from 17 to 87 years, with a median age of 47. Tuberculin skin tests were positive in 42 of the 48 patients. The time from the onset of pleurisy to biopsy was 1 to 12 months with a median of 3 months for needle biopsies and 2 to 8 months with a median of 4 months for open biopsies. There was evidence of parenchymal pulmonary disease by chest roentgenogram in 5 of the 13 patients with surgical biopsies, and in 21 of the 42 patients with needle biopsies. Pleural effusions were present in 12 of the 13 patients with surgical biopsies, and in 33 of the 42 patients with needle biopsies.

The techniques of Vim-Silverman needle biopsy of the pleura (DeFrancis, Klosk and Albano[5], Heller, Kellow and Chomet[12]) and of limited surgical biopsy of the pleura (Breckler *et al.*[3]) have been well described in the past. In our experience, the best results with needle biopsy have been obtained with the following technique. Fluid should first be removed for examination to avoid blood-staining secondary to the needle biopsy. This also permits accurate localization of the parietal pleura. During the initial biopsy, it is possible to obtain multiple samples by directing the biopsy needle at different angles, thus enhancing the possibility of obtaining diagnostic tissue. The absence of pleural fluid does not represent an absolute contraindication, but a thickened parietal pleura is then essential. The core of tissue obtained with the Vim-Silverman needle is most easily removed by placing the split needle in saline and gently teasing the specimen loose to avoid crush artefact.

Results. NEEDLE BIOPSY. The histologic diagnoses made from the needle biopsies are shown in Table 1. Diagnoses were established in 26% of the patients, non-specific chronic pleuritis was found in 45% of the patients, and in 29% of the patients no pleura was obtained. Acid-fast bacilli were demonstrated in 2 of the 5 specimens with granulomatous pleuritis. All of the 5 patients with granulomatous pleuritis responded well to antituberculous therapy. Chronic pleuritis was defined as non-specific thickening and infiltration of the pleura with chronic inflammatory cells.

The diagnosis of eosinophilic granuloma was later shown to be incorrect at thoracotomy. The patient was a 50-year-old man with a pleural effusion and eosinophilia. Examinations of the fluid revealed an exudate with negative bacteriologic, parasitic, and cytologic studies. The eosinophil count in the pleural fluid ranged from 50 to 82%.

TABLE 1.—NEEDLE BIOPSY OF THE PARIETAL PLEURA IN 42 PATIENTS

Pathologic Diagnosis	*Patients*	*%*
Granuloma	5	12
Malignancy	3	7.1
Leukemia	1	2.3
Eosin. Granuloma	1*	2.3
Mycotic Infect.	1	2.3
Chronic Pleuritis	48	45
Inadequate	12	29
TOTAL	42	100

*Later shown to be a malignancy.

A needle biopsy of the pleura demonstrated what was felt to be an eosinophilic granuloma. Bronchoscopy subsequently revealed a bronchogenic carcinoma of the right main stem bronchus. At thoracotomy the parietal and visceral pleura were studded with tumor which microscopically revealed nests of malignant cells surrounded by an intense eosinophilic reaction. This case was so unusual that it has been described elsewhere in detail (Majumdar and Zahn[18]).

Although only 11 diagnostic needle biopsies were obtained, they were the only technique by which a diagnosis was established in 9 of these 11 patients. In 15 of the 31 patients with inconclusive needle biopsies (either non-specific chronic pleuritis or inade-

quate specimens), the etiology of the effusion was finally determined by other methods. Pleural fluid cultures were positive in 6 patients (3 tuberculous and 3 non-tuberculous). Sputum cultures grew out tubercle bacilli in 3 patients. Pleural fluid cytology revealed malignant cells in one patient. Surgical biopsy of the pleura demonstrated tuberculosis in 2 patients, carcinoma in 2 patients, and histoplasmosis in one patient. Thus, the results of needle biopsy were as good as, or better than, any other single examination with the exception of surgical biopsy.

TABLE 2.—FINAL DIAGNOSIS IN 31 PATIENTS WITH CHRONIC PLEURITIS OR INADEQUATE TISSUE OBTAINED BY NEEDLE BIOPSY

Tuberculous pleuritis	8
Presumed tuberculous pleuritis	12
Malignancy	3
Non-tuberculous empyemas	3
Histoplasmosis	1
Uncertain	4

As is shown in Table 2, the findings of non-specific chronic pleuritis or inadequate tissue on needle biopsy did not rule out serious underlying disease. Tuberculosis was proven in 8 of these patients, and malignancies were found in 3 patients. The 12 patients with presumed tuberculous pleuritis all had a positive tuberculin skin test and responded well to antituberculous drugs. In the 3 patients with non-tuberculous empyema, there was no evidence of underlying disease after careful evaluation, and all 3 responded well to appropriate antibiotic therapy. The etiology of the effusion could not be determined in 4 patients even after surgical biopsy.

The only major complication in the 42 patients who had needle biopsies performed was a partial pneumothorax, requiring catheter aspiration in one patient. One other patient developed a small pneumothorax which spontaneously reabsorbed. Although lung tissue was found in three specimens, intercostal bone marrow in one specimen, and liver in one specimen, there were no other complications.

The following case reports illustrate the clinical application of the procedure.

Case Reports. CASE 1. A 41-year-old woman with a positive tuberculin skin test was admitted from an outside hospital with a right pleural effusion of uncertain etiology. Chest roentgenogram revealed a right pleural effusion (Fig. 1*A*) which was found to be an exudate. A needle biopsy of the pleura conducted 2 days after admission revealed granulomatous lesions compatible with tuberculosis (Fig. 1*B*). Under treatment with antituberculous drugs and adrenal corticosteroids, the effusion promptly cleared, and the patient returned to work in 6 months. All sputum samples, pleural fluid cultures, and gastric washings were negative for tubercle bacilli throughout her hospitalization.

CASE 2. A 44-year-old man with a positive tuberculin skin test was admitted with a 2-month history of right-sided pleurisy with effusion. Chest roentgenogram demonstrated a right pleural effusion (Fig. 2*A*). The pleural fluid was an exudate with negative cytologic and bacteriologic findings. A needle biopsy of the pleura performed one week after admission revealed anaplastic carcinoma invading the parietal pleura (Fig. 2*B*). The diagnosis was subsequently confirmed by surgical biopsy of the pleura. Examinations for a primary site were unsuccessful, and the patient was discharged to receive palliative care.

CASE 3. A 60-year-old man, former miner, was admitted with a history of dyspnea on exertion and abnormal chest roentgenograms of 15 years' duration. Numerous studies at other hospitals had failed to demonstrate tubercle bacilli, and he had been considered to have silicosis. Chest roentgenograms over a period of 10 years demonstrated the progressive development of either large bullae or cavities in both apical areas (Fig. 3*A*). A needle biopsy of the thickened pleura adjacent to the left apical cavity demonstrated large colonies of fungi, which microscopically resembled *Aspergillus* (Fig. 3*B*). Cultures of sputum, gastric washings, bronchoscopic washings, and a scalene lymph node were all neg-

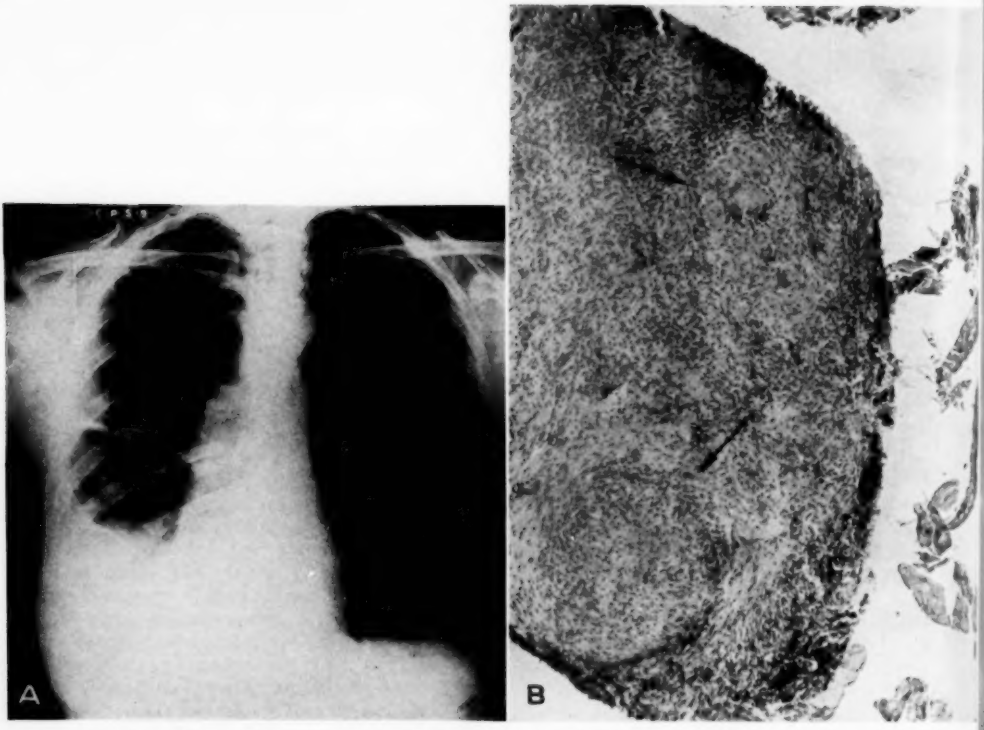

Fig. 1.—*A*, Chest roentgenogram of Case 1. *B*, Needle biopsy of Case 1, demonstrating two granulomas in the parietal pleura, one containing a Langhans' giant cell. Hematoxylin and eosin × 160.

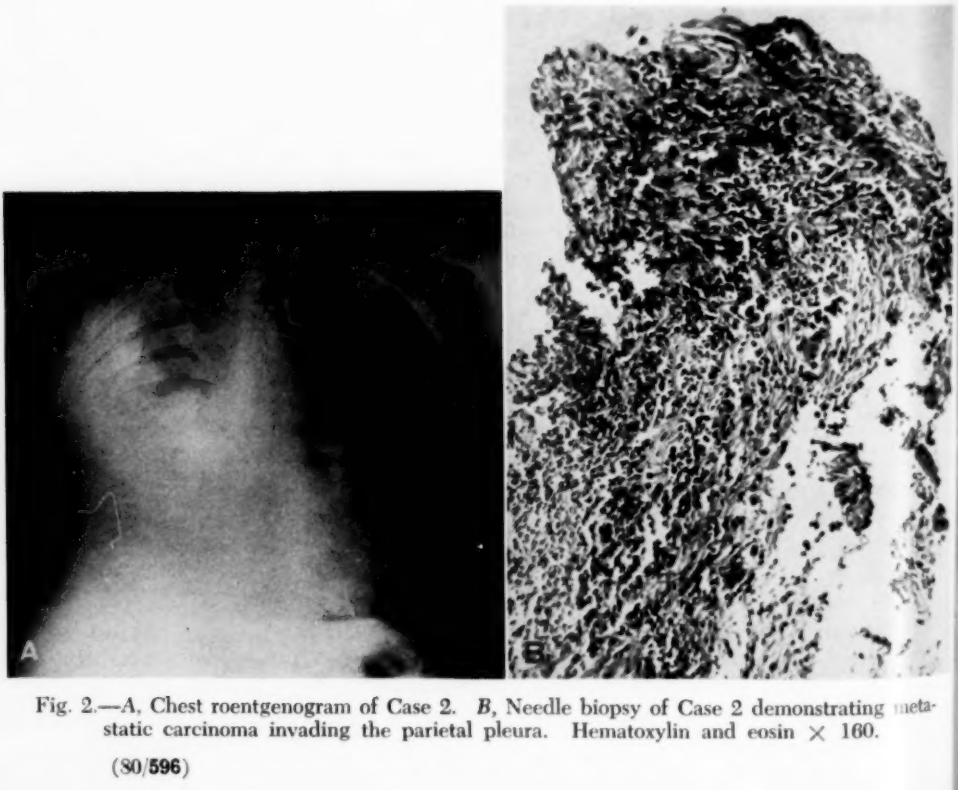

Fig. 2.—*A*, Chest roentgenogram of Case 2. *B*, Needle biopsy of Case 2 demonstrating metastatic carcinoma invading the parietal pleura. Hematoxylin and eosin × 160.

ative. Under treatment with sulfonamides and iodides, he has shown slow improvement.

SURGICAL BIOPSIES. The results obtained with surgical biopsies of the parietal pleura are shown in Table 3. Exploratory thoracotomies were undertaken in 6 patients, and 7 patients had limited thoracotomies under intercostal nerve block anesthesia. As might be expected, the yield from surgical biopsy was greater than that of needle biopsy. Granulomatous tuberculous pleuritis was proven in 3 patients when all other methods, including needle biopsy in 2 patients, had failed. Metastatic carcinoma was diagnosed in 2 patients and confirmed in a third patient with a needle biopsy revealing neoplastic invasion (Case 2). Even at thoracotomy, non-specific chronic pleuritis was the only finding in 7 patients of whom 4 had never received antituberculous drugs.

COMMENTS. The previously reported needle and surgical biopsies of the parietal pleura are summarized in Tables 4 and 5. In an attempt to avoid duplication of cases, only the largest series of patients reported by an author have been included. Lymphomas and leukemia have been included under the general term of malignancy. The results obtained in different studies often depend on the type patient under ob-

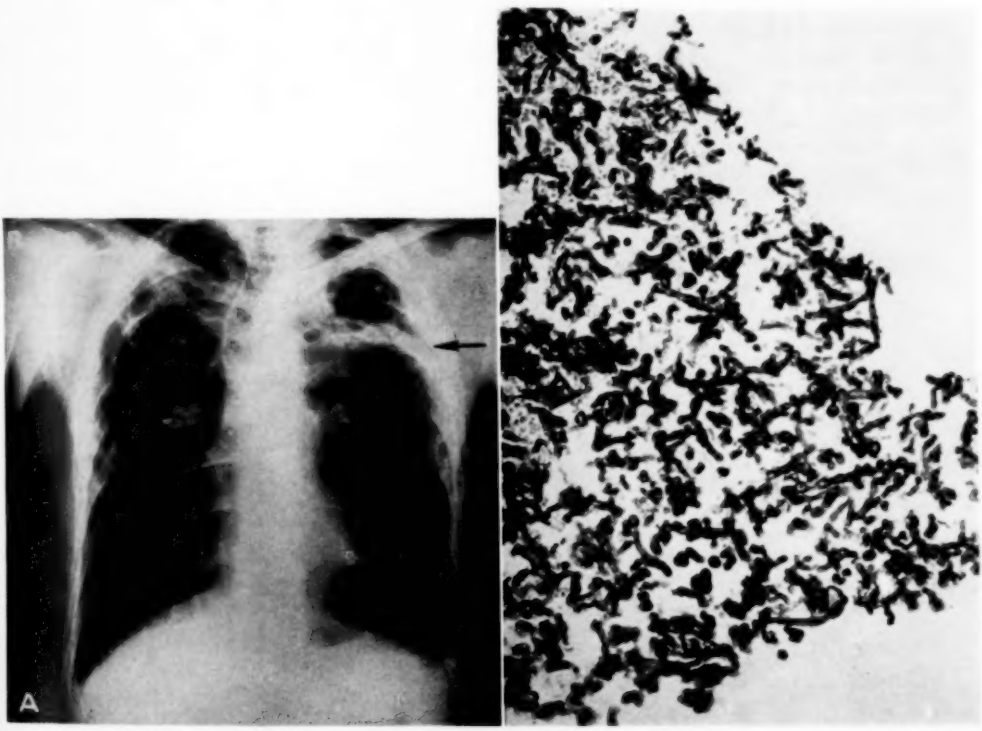

Fig. 3.—*A*, Chest roentgenogram of Case 3 demonstrating pulmonary fibrosis, emphysema, and bilateral apical cavities. Needle biopsy was performed at the site indicated by the arrow. *B*, Needle biopsy of Case 3 demonstrating branching septate hyphae with spore formation. In other areas, the hyphae directly invaded the parietal pleura. Periodic acid Schiff × 480.

servation. For example, Weiss[32] studied needle biopsies in patients with tuberculosis, while Samuels, Old and Howe[25] were evaluating needle biopsies in malignant pleural effusions. Other authors have utilized pleural biopsies in diverse pleural diseases.

TABLE 3.—SURGICAL BIOPSY OF THE PARIETAL PLEURA IN 13 PATIENTS

Pathologic Diagnosis	*Patients*	*%*
Granuloma	3	23
Malignancy	3	23
Chronic Pleuritis	7	54
TOTAL	13	100

sies were seldom undertaken. The frequency of successful diagnostic biopsies tended to increase with the number of specimens obtained. Finally, several of the needle biopsies were performed months after the administration of antituberculous drugs, perhaps masking the nature of the lesion.

In view of the better results obtained with surgical biopsy of the pleura, some investigators have gone directly to open biopsies. Surgical biopsy of the pleura has the advantage of yielding a larger specimen with more frequent histological diagnoses. Portions may also be cultured. Nevertheless, it requires the presence of a thoracic surgeon and the use of an operating room fully equipped for endotracheal anesthesia. To reduce morbidity, the procedure often has to be delayed until after the acute pleuritis has subsided. Furthermore, although the surgical procedure is a relatively minor one, there is postoperative morbidity and considerable additional expense. Finally, surgical biopsy may be obviated if a diagnosis is obtained by the simpler and less traumatic technique of needle biopsy.

TABLE 4.—SUMMARY OF PREVIOUSLY REPORTED NEEDLE BIOPSIES OF THE PARIETAL PLEURA

Authors	*Patients*	*Granuloma*	*Malignancy*	*Chronic Pleuritis*	*Normal*	*Inadequate*
DeFrancis *et al.*[5]	6	2	0	0	0	4
Heller *et al.*[13]	45	6	8	28	0	3
Donohoe *et al.*[7]	78	22	8	29	1	18
Weiss[32]	31	23	0	0	0	8
Mestitz *et al.*[19]	200	71	33	92	0	4
Samuels *et al.*[25]	52	0	25	7	0	20
Welsh[33]	17	0	10	2	0	5
Leggat[15]	20	0	12	7	0	1
Misra and Sharma[20]	49	5	1	39	4	0
Green[11]	18	4	5	3	0	6
Richert *et al.*[22]	23	6	0	8	0	9
TOTALS	539	139	102	215	5	78

The number of diagnostic biopsies obtained by needle aspiration in the present study is less than that obtained in some earlier reports. Several factors would seem to explain these results. First, the needle biopsies were performed by several physicians. As experience was gained with the procedure, better specimens were more frequently obtained. Second, 9 of the 42 patients had no free pleural fluid present, making pleural localization difficult. Third, in the 31 patients with non-specific chronic pleuritis or inadequate specimens, repeat needle biop-

Needle biopsy of the pleura has sev-

eral advantages. It is a simple technique with no more morbidity than a standard thoracentesis. During a thoracentesis, the only additional equipment required is a biopsy needle. It permits the early diagnosis of effusions and may represent the only means by which an etiologic diagnosis is established. A positive diagnosis is very reliable. Including our patient, there have been only two incorrect pathological diagnoses made from needle biopsies of the pleura (Mestitz, Purves and Pollard[19]).

unexplained pleural effusions after careful study, including needle biopsy (Donohoe, Katz and Matthews[7], Richert *et al.*[22], Thomas and Anderson[30]).

In addition to our 7 patients, there have now been a large number of patients described in whom only non-specific chronic pleuritis has been found at surgical biopsy (Table 5). The consistent demonstration of this finding has led some investigators to postulate the existence of idiopathic benign pleural effusions, similar to idiopathic benign pericarditis. Indeed, Coxsackie

TABLE 5.—SUMMARY OF PREVIOUSLY REPORTED SURGICAL BIOPSIES OF THE PLEURA*

Authors	*Patients*	*Granuloma*	*Malignancy*	*Chronic Pleuritis*	*Miscellaneous*	*Normal*	*Inadequate*
Lloyd[17]	16	0	12	0	0	0	4
Sutliff *et al.*[29]	21	17	2	1	1	0	0
Small and Landman[26]	5	5	0	0	0	0	0
Heller *et al.*[13]	5	2	0	2	1	0	0
Douglass *et al.*[8]	21	5	6	7	3	0	0
Hill *et al.*[14]	50	17	2	25	6	0	0
Donohoe *et al.*[7]	54	23	5	21	0	4	1
Mitchell *et al.*[21]	25	8	0	10	7	0	0
Smith *et al.*[27]	25	17	1	6	0	1	0
Thomas and Anderson[30]	15	6	0	9	0	0	0
Richert *et al.*[22]	37	19	0	18	0	0	0
TOTALS	274	119	28	99	18	5	5

*Includes thoracoscopy, limited pleural biopsy, and full thoracotomy.

The importance of further evaluating patients with chronic pleuritis or inadequate specimens on needle biopsy should be emphasized. In addition to the present series, there have been several reports of tuberculosis, malignancy, or other pleural diseases eventually discovered in such patients (Donohoe, Katz and Matthews[7], Mestitz, Purves and Pollard[19], Richert *et al.*[22], Samuels, Old and Howe[25], Weiss[32]). It has now become standard policy at several centers to perform a limited surgical biopsy of the pleura in all patients who continue to have

virus infections have been shown to produce both pleurisy with effusion and pericarditis (Gordon, Lennette and Sandrock[10]). Initially, it was possible to object to this postulate as the patients had often received antituberculous drugs for several months before surgery (Douglass, Carr and Bernatz[8], Small and Landman[26], Stead, Eichenholz and Stauss[28], Sutliff, Hughes and Rice[29]). However, non-specific chronic pleuritis has now been repeatedly found at open pleural biopsy in patients uncomplicated by drug treatment (Donohoe, Katz and Matthews[7],

Hill, Hensler and Brackler[14], Mitchell *et al.*[21], Richert *et al.*[22], Smith, Parsons and Daniels[27], Thomas and Anderson[30]). At some centers such patients are now being considered nontuberculous and discharged without treatment (Donohoe, Katz and Matthews[7], Richert *et al.*[22], Thomas and Anderson[30]).

Obviously, pleural biopsies do not replace standard methods of examining pleural effusions. In many cases, bacteriologic and cytologic studies will provide an answer. Pleural biopsies augment these procedures and may be diagnostic when other methods have failed. In cases of tuberculous pleuritis needle biopsies may prove the diagnosis in days, while cultures require weeks for confirmation. By the addition of pleural biopsy to the diagnostic tools now available, the number of "idiopathic pleural effusions" and "presumed tuberculous pleural effusions" should show a considerable decrease.

Summary and Conclusions. 1) The results of needle biopsies of the parietal pleura in 42 patients and surgical biopsy of the pleura in 13 patients are reported. Diagnostic tissue was obtained in 26% of the needle biopsies and 46% of the surgical biopsies. There was one incorrect diagnosis made from a needle biopsy.

2) Needle biopsy of the parietal pleura is a safe, rapid, and accurate method of establishing an etiologic diagnosis in pleural effusions. The routine use of this technique at the time of the initial thoracentesis is recommended.

3) The finding of non-specific chronic pleuritis by needle biopsy does not eliminate the possibility of underlying tuberculosis or malignancy. If a diagnosis cannot be otherwise obtained, surgical biopsy of the pleura is recommended.

4) Pleural biopsy augments, but does not replace, standard methods of examining pleural effusions.

5) The diagnoses of "presumed tuberculous pleural effusions" and "idiopathic pleural effusion" no longer seem justified, in view of the ease and availability of pleural biopsy.

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SUMMARIO IN INTERLINGUA

Biopsia del Pleura Parietal

1. Es reportate le resultatos de biopsia a agulia in le pleura parietal de 42 patientes e de biopsia chirurgic in le pleura de 13 patientes. Tissu de valor diagnostic esseva obtenite in 26% del biopsias a agulia e in 46% del biopsias chirurgic. Esseva facite un diagnose incorrecte super le base de un biopsia a agulia.

2. Biopsias a agulia in le pleura parietal es salve, rapide, e accurate como methodo de establir un diagnose etiologic in effusion pleural. Le uso routinari de iste technica al tempore del thoracentese initial es recommendate.

3. Le constatation de non-specific pleuritis chronic per biopsia a agulia non elimina le possibilitate de un subjacente tuberculose o malignitate. Si le diagnose non pote esser obtenite per altere mesuras, biopsia chirurgic del pleura es recommendate.

4. Biopsia pleural es un adjuncto e non un reimplaciamento del methodos standard pro le examine de effusiones pleural.

5. Le diagnoses de "presumite effusiones pleural tuberculotic" e "idiopathic effusiones pleural" pare haber perdite lor justification, viste le disponibilitate e le facilitate de biopsia pleural.

SUMMARIO IN INTERLINGUA

(See page 602 for original article)

Un Manometro "a Uso Unic" pro Determinationes del Tension Venose

Un manometro "a uso unic" pro mesurationes del tension venose es describite. Illo es construite ex un commercialmente disponibile tubage plastic e es montate in un supporto etiam de plastico. Le production e le uso del manometro es describite in detalio. Varie observationes es addite con respecto al technica del mesuration de tensiones venose.

DISPOSABLE MANOMETER FOR VENOUS PRESSURE*

By Capt. H. A. Schlang, MC, USN

and

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In the course of planning a study of venous pressure in a number of pregnant individuals, an attempt was made to simplify the usual venous pressure tray. The latter, in our hospital, contains a spinal manometer in two sections, with an array of needles, syringes, stopcocks and draping. An apparatus was developed using the usual sterile needle, syringe and three-way stopcock, each individually packaged and therefore interchangeable, plus a disposable manometer held firmly in a transparent holder on a laboratory stand. The manometer itself is a commercially available sterile plastic tube ("Venotube-20")† 20 inches long, which is stretched into place in the manometer holder and then serves as its own flexible connection to the stopcock.

The manometer holder is made of ¾-inch-thick lucite plate, 2 inches wide by 8 inches long. A longitudinal slot ⅛ inch wide by ¼ inch deep is made in the long centerline by cutting on a table saw. A suitable scale in millimeters is attached to the back of the holder. Four holes are drilled in the corners for attachment by rubber bands or other fixation to the laboratory stand. A piece of pipe cut to proper length or a laboratory clamp affixed to the stand provides a firm base at a fixed level above the tabletop for the bottom (and 0 point) of the manometer holder.

In the actual procedure of determining venous pressure, the female end of the Venotube-20, which has a firmly attached hub, is pressed into the upper end of the slot. The hub then sits firmly on the top edge of the holder. With slight stretching, the plastic tube is forced into place throughout the length of the slot, providing a perfectly straight manometer tube. The press-fit holds the tube snugly and it is not dislodged by the minor manipulations of the free portion of the tube when it is attached to the side arm of the stopcock (Fig. 1).

After the Venotube-20 is in place in the manometer holder, a sterile 10-ml. syringe, stopcock, and nineteen-gauge needle are connected in line. A few milliliters of concentrated heparin are aspirated into the syringe and then expelled back into the vial, leaving a small residual in the syringe and needle. Five milliliters of sterile isotonic saline are next aspirated into the syringe. The sterile male end of the Venotube-20 is uncapped and placed in the side arm of the stopcock. The manometer is then filled to the 200-mm. mark and the stopcock aligned in the syringe-needle direction. The antecubital veins are distended by the application of a tourniquet and cleansed

*The opinions given are those of the authors and are not necessarily those of the Naval Service at large.

†Manufactured by Abbott Laboratories, North Chicago, Illinois.

with an alcohol sponge. The venous pressure is measured in the usual fashion after insertion of the needle and release of the tourniquet.

The procedure is carried out on a flat and level surface, sufficiently wide to permit 60° abduction of the arm. A pillow is placed under the head, but the back is flat on the table. No padding other than a layer or two of sheeting is provided. The manometer on its stand is placed conveniently near the arm. The bottom of the holder (0 point) is arbitrarily set at 10 cm. above the table top. We have found it of interest to measure the depth of the chest at the fourth costochondral junction, with the ordinary Roentgen-ray chest caliper, so that the antero-posterior diameter of the chest may be included in the interpretation of the venous pressure reading.

The disposable manometer described above has the advantage of ready availability, since all parts may be carried easily in a physician's bag.

Although the spinal manometers usually employed for venous pressure are reusable, breakage is likely and the initial cost is approximately twenty times that of the unbreakable, disposable manometer*. Further, the spinal manometer needs careful cleansing, packaging and autoclaving after each usage, requiring time, space and material. Lastly, there is the elimination of the risk, remote though it may be, of the transmission of serum hepatitis by the manometer tube, since the venotube is used only once.

Summary. A disposable venous pressure manometer made of commercially available plastic tubing and mounted in a plastic holder is described. Details of construction and use are given with some comment on the procedure of measuring venous pressure.

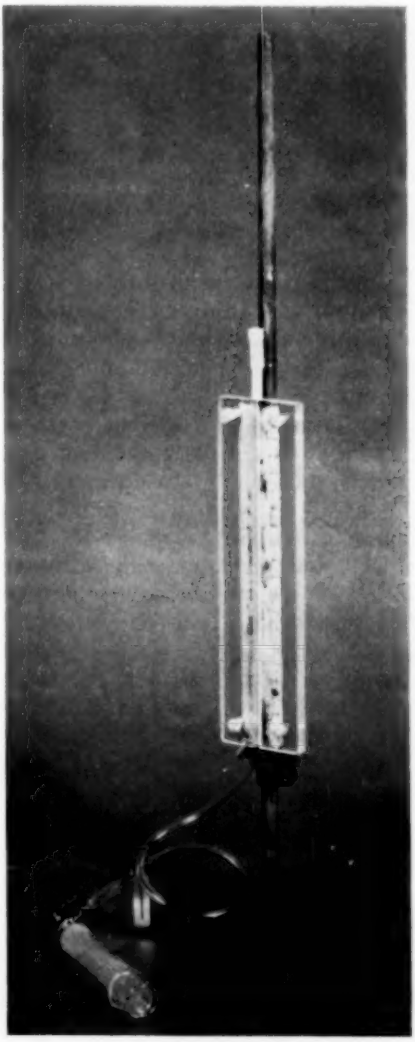

Fig. 1

*The cost of the holder is nominal. It can be produced readily in any small carpenter shop. Each "Venotube-20" is available commercially for 28¢; the long 30-inch version costs 30¢.

(Interlingua on page 601)

STUDIES OF OPPORTUNISTIC FUNGI.
I. INHIBITION OF *RHIZOPUS ORYZAE* BY HUMAN SERUM

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A recent revival of interest has occurred in the study of substances in normal human serum which are inhibitory to pathogenic microorganisms; among the fungi, these have been limited primarily to studies of factors inhibitory to dermatophytes. Lorincz, Priestley and Jacob[7] have demonstrated a general occurrence in human and mouse serum of a dialyzable, heat labile, antidermatophytic substance. Roth and his colleagues[11] report that the substances inhibiting dermatophytes differ from those affecting *Candida albicans*, in that the former are dialyzable, heat labile, and present from birth, whereas the latter are relatively heat stable and virtually absent from birth up until 3 to 6 months of age.

Jessner and Hoffman[6] seem to have been the first to report on the fungistatic activity of blood serum. Per and Braude[9] followed shortly thereafter with a report on the therapeutic value of trichophytin in dermatophytosis. They found that parasitic fungi ceased growing within 2 days after being mixed *in vitro* with serum from allergic patients (presumably patients showing dermatophytids). After injection with trichophytin, serum from dermatophytid patients ceased to be an effective inhibitor of dermatophytic fungi.

Ayres and Anderson[1] believed that they had demonstrated circulating fungicidal antibodies in the serum of patients who had phytid lesions when they were unable to obtain growth of dermatophytes on Sabouraud's agar medium containing 8% serum from such patients. They found no inhibition at this concentration using either normal serum, or serum from patients with dermatophytosis without dermatophytid lesions. Peck, Rosenfeld and Glick[8] were unable to duplicate these results, and concluded that although blood serum may be fungistatic, there was no increase in fungistasis by serum from patients with dermatophytids as compared with normal controls. The activity of serum from individuals with no history of dermatophytosis varied from slight to strong, and within the same individual there appeared to be a seasonal variation in activity. Heating for 20 minutes at 56° C. appeared to increase fungistatic activity, but with prolonged heating, activity generally decreased.

Blank[4] and Roth[11] with their coworkers, found that on Sabouraud agar containing 50% serum, mycelial pellets of certain dermatophytes at first remained stationary, and finally began a slow growth after 96 hours or more. Transplants made at any time during

this lag phase developed normal colonies rapidly, thus indicating that serum is fungistatic rather than fungicidal. The prolonged lag phase suggests that the inhibiting factors in serum may be metabolized by the organism or else decompose spontaneously over a period of several days. The observations that dialysis and heating reduced but did not destroy fungistatic activity, and that certain purified protein fractions including gamma globulin were inactive, suggest that several factors inhibit dermatophytes. These factors seem to be different from those inhibiting *Candida albicans*.

This report is concerned with the effect of serum on an opportunistic fungus, *Rhizopus oryzae*. This fungus is frequently found as the etiologic agent in mucormycosis, a secondary and usually rapidly fatal infection in patients with certain predisposing diseases (Baker[2], Hutter[5]).

Materials and Methods. The test organism, *Rhizopus oryzae*, was obtained through Dr. Ernest Chick. It had been isolated originally from a fatal case of cerebral mucormycosis by Bauer *et al.*[3]. Human serum was obtained from routinely qualified donors of blood at the Duke Hospital Blood Bank. These sera gave results quite similar to those obtained from pooled sera derived from 20 to 40 unselected patients at the Durham Veterans Administration Hospital. Human umbilical cord serum was obtained from the Duke Hospital Serology Laboratory to which specimens are sent directly from the delivery rooms for routine serologic tests. All sera were separated from the clots by centrifugation after retraction and stored at −20° C. until used.

Fifty milliliter Erlenmeyer flasks were used as culture vessels in all tests. To each flask was added 4.0 ml. Sabouraud liquid medium prepared at five times the routine concentration. When brought to a final volume of 20 ml. with diluents, each flask then contained the ordinary concentration of Sabouraud medium. In order to obtain a final volume of 20 ml. per flask, a diluent of 0.9% saline with Na-K Phosphate buffer of pH 7.4 in a final concentration of 0.005M was used throughout. Preliminary determinations showed, however, that the growth response of the test organism to increasing serum concentrations was little influenced by the diluent be it this buffer, distilled water, or unbuffered saline. This similarity of response with the three diluents was interpreted as evidence of an effect of serum *per se* rather than an artifact of the particular diluent employed.

Aseptic technique was used in all tests. Certain sera were available only after possible contamination, therefore one drop of 5% chloramphenicol was added to each culture flask. This concentration of the antibiotic was without effect on the growth of the test organism and prevented growth of bacterial contaminants during the incubation period.

Spore suspensions were prepared from 4 to 8-day-old cultures of *R. oryzae* on Sabouraud agar in screw-capped culture tubes by adding 5.0 ml. sterile distilled water and manipulating with a sterile glass rod. Centrifugation of an aliquot of this suspension in an hematocrit tube generally revealed a spore concentration of approximately 0.5%. In a preliminary experiment to determine the influence of spore concentration on the amount of growth obtained at the end of a specific interval, 1.0 ml. volumes of spore suspensions prepared at varying percentages (V/V) were added to 19.0 ml. Sabouraud broth prepared at routine concentration. Inspection of the curve of response showed that the greatest variation occurred as the concentration of the spore suspension was increased from 0.001 to 0.003%. Above this latter concentration, there was little more increase in growth as the concentration of inoculum was increased. Consequently 1.0 ml. of a 0.005% spore suspension was used as inoculum in all tests. In a separate experiment to determine the influence of spore concentration on the degree of inhibition produced by a given concentration of serum, it was found that the effects of serum were unaltered by 0.001 to 0.05% spore concentration in a 1.0 ml. inoculum.

Flasks containing the mixture of Sabouraud broth, saline and chloramphenicol were autoclaved. After they had cooled to room temperature, serum was added followed by 1.0 ml. inoculum. After 18 to 20 hours incubation at room temperature on a reciprocating shaker (100 complete strokes per minute), the medium was removed by filtering through eight layers of gauze. The retained mycelial pellets were washed with approximately 25 ml. distilled water, blotted briefly on filter paper and dried at 75° C. for 18 hours on tared #2 coverslips. The dry weight was determined to the nearest 0.1 mg. Replicate

determinations showed the average per cent deviation from the mean to be <5% when the spore suspension had been thoroughly mixed prior to pipetting each aliquot.

Results. The growth response of *R. oryzae* to increasing concentrations of normal adult serum was biphasic; an initial increase in growth at 5% to 10% serum concentration was followed by a decrease, with virtually complete inhibition occurring between 20% and 30% serum. Figure 1(*A*) is a composite plot of this response with five separate pools of serum, each pool containing a somewhat lower concentration (Fig. 1 *[B]*). Certain pools of cord sera gave results quite similar to normal adult serum in the stimulatory phase; others manifested very slight stimulation at the lower concentration.

That inhibition was not due merely to a change in hydrogen ion concentration was shown by inoculating flasks containing Sabouraud broth alone which had been adjusted with concentrated sodium hydroxide to the same pH as those flasks containing serum. No variation in growth occurred over

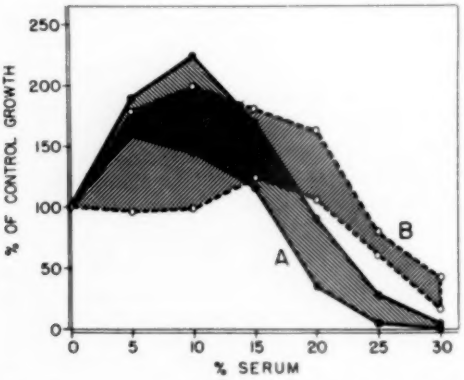

Fig. 1.—Composite graph of inhibition of *R. oryzae* by (*A*) pooled adult serum and (*B*) pooled human umbilical cord serum. The dry weight obtained is expressed as % of the dry weight from the control flask with no serum and is plotted against % serum concentration in the culture flask.

from 5 to 40 individual samples. Comparing activities of normal donor sera with pools obtained from unselected patients from medical, surgical and psychiatric services, the only difference noted was an occasional slight depression of the increased growth phase when using patients' sera. When this occurred, complete inhibition of the test organism was frequently obtained at a lower serum concentration than was obtained with serum from normal, unfasted donors.

The inhibitory factors were present in human umbilical cord serum, but in a pH range of 6.4 to 7.1, which encompassed the pH range of the experimental flasks containing 0% to 30% serum.

Heating serum at 56° C. for up to 2 hours depressed the stimulatory phase of the response curve at lower serum concentrations but was without effect on the degree of inhibition obtained with 25% serum (Fig. 2). Serum which had been diluted 1:1 with buffered saline and heated at 80° C. for 15 minutes yielded a yellowish supernatant solution after centrifugation at 18,000 × g for 30 minutes. When this

was assayed, correcting for the dilution factor, it was found that inhibition still occurred, but was not as great as in untreated serum. Figure 3 shows this graphically; this figure also shows failure to obtain inhibition after treating normal serum with activated charcoal followed by filtration through precipitate was noted when dialysis was accomplished against buffered saline. Figure 4 shows the results of assays after dialysis under each of these two conditions. The stimulatory portion of the curve is actually exaggerated, indicating that stimulation at lower serum concentrations is due to

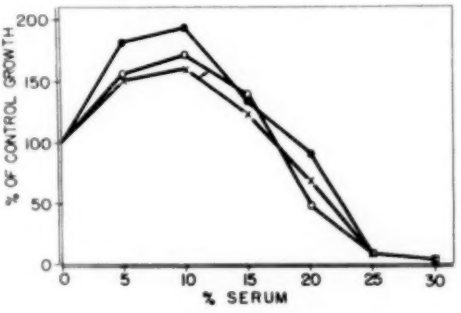

Fig. 2.—Influence of prior heating of serum at 56° C. on its effect on the growth of *R. oryzae*. Open circles, heated 30 minutes; crosses, heated 2 hours; closed circles, unheated control. Coordinates same as in Fig. 1.

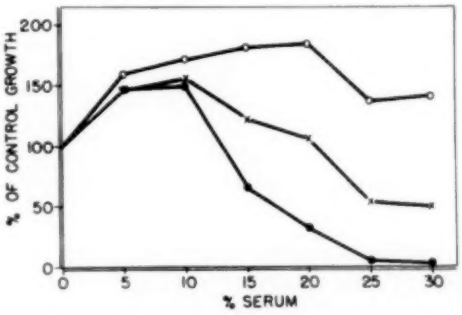

Fig. 3.—Influence of (crosses) heating serum to 80° C. for 15 minutes and (open circles) treatment of serum with charcoal on its effect on the growth of *R. oryzae*. Closed circles, untreated serum. Coordinates same as in Fig. 1.

Whatman No. 40 paper. Passage of serum through a Seitz filter pad (Hercules Filter Corp., Paterson, N.J., type ST) was without effect on the growth response.

Dialysis against glass-distilled water (tubing from Visking Co., Chicago, Ill.) at 5° C. for 24 hours yielded a fine precipitate in the dialysis bags; no high molecular weight, non-diffusible substances. The factors responsible for inhibition are removed by this treatment. Removal of properdin from serum by adsorption on inulin and zymosan failed to affect the degree of inhibition obtained. Purified lysozyme (Nutritional Biochemicals Co.) in saline solution was not inhibitory at

concentrations equivalent to those in normal serum. Assay of a globulin fraction, obtained by adding ammonium sulfate to serum to a final concentration of 40% of saturation followed by high speed centrifugation and re-dissolving the precipitate in buffered saline to a concentration equivalent to that in normal serum, showed an initial stimulation of growth at lower concentrations quite similar to untreated serum. This stimulation, however, persisted through an equivalent serum concentration of 30%; no inhibitory phase was demonstrated. Serum which had been incubated 1 hour at room temperature with mycelial pellets grown in Sabouraud broth alone, then separated from the pellets by filtration, showed no alteration of the inhibitory pattern upon assay.

To determine if recovery from inhibition at a given serum concentration would occur, a series of flasks containing 0% to 35% serum at 5% increments was set up in triplicate. A series was harvested at 18 hours, another at 42 hours, and the last at 66 hours. Figure 5 shows the results. At 42 hours, there was little or no inhibition in the flask containing 30% serum, but inhibition was still virtually complete in 35% serum. At 66 hours even inhibition in 35% serum was largely overcome and growth exceeded that in Sabouraud broth without serum.

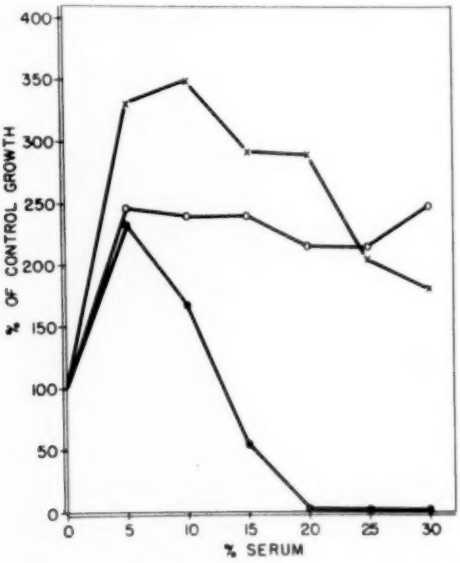

Fig. 4.—Influence of dialysis of serum against (open circles) glass-distilled water and (crosses) buffered saline on its effect on the growth of *R. oryzae*. Closed circles, untreated serum. Coordinates same as in Fig. 1.

In view of evidence of at least one factor in normal serum inhibitory to the test organism, it was considered of some practical interest to determine if serum from patients with a predisposing disease would yield an altered pattern of inhibition. From each of 6 patients with known diabetes mellitus, sufficient serum (22 ml.) was obtained to perform the complete assay from 0

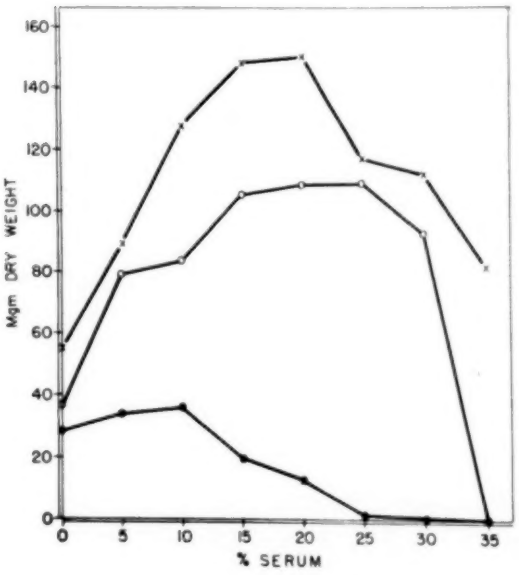

Fig. 5.—Escape by *R. oryzae* upon prolonged incubation from serum-induced inhibition. Closed circles, open circles and crosses were harvested at 18, 42 and 66 hours, respectively. Dry weights obtained are plotted against serum concentrations.

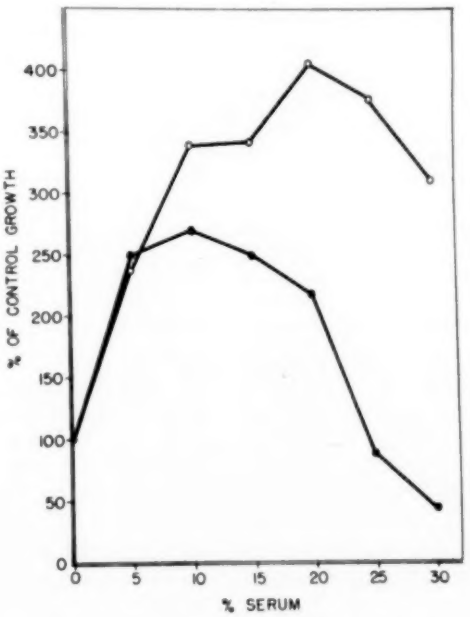

Fig. 6.—Closed circles, titer of normal serum; open circles, titer of serum from L.V.H. See text for clinical summary. Coordinates same as in Fig. 1.

to 30% serum at 5% increments. Of these 6, definite departures from the usual pattern were obtained with 2. The other 4 were not in acidosis at the time the serum was drawn. The two altered curves are shown in Figs. 6 and 7; a summary of the clinical history of each patient follows:

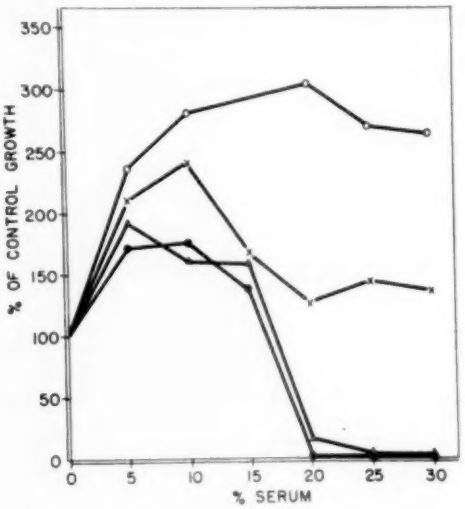

Fig. 7.—Closed circles, titer of normal serum; open circles, crosses and triangles, titers of serum from J.H.G. on admission, and at 15 hours and 6 days after admission, respectively. See text for clinical summary. Coordinates same as in Fig. 1.

Case Reports. CASE 1. L.V.H., 46-year-old white male, was admitted dead on arrival at the Durham Veterans Administration Hospital on December 22, 1960. He had had diabetes mellitus for a period of 15 years, controlled with 30 units PZI insulin each day. He was first seen at this hospital in May, 1959, at which time a diagnosis of carcinoma of the prostate was made. At that time a bilateral orchiectomy was performed and estrogen therapy started. He was admitted to this hospital three more times prior to death, and on one admission received 6,400 r. over the right humerus, left hip and spine for pain due to metastatic disease. He did fairly well until 3 months prior to death at which time he developed ankle edema with orthopnea. On the fourth admission in October, 1960, he had mild congestive failure. A roentgenographic bone survey at that time revealed extensive osteoblastic metastases scattered through the dorsal lumbar spine, pelvis, proximal portion of the left femur and through numerous ribs. He was digitalized and discharged on November 17, 1960. No history was available of terminal events of his death. A necropsy was performed approximately one hour after death and revealed widespread metastases in the liver, lungs, bones, gastrointestinal tract, prostate and adrenals. Blood was taken from the inferior vena cava for antifungal assay.

CASE 2. J.H.G., 45-year-old colored male, was admitted to the Durham Veterans Administration Hospital at 10:00 a.m. on December 28, 1960, in diabetic acidosis. On admission, blood glucose was 336 mg. per 100 ml., the CO_2 capacity was 8.8 mMoles per L., the test for ketone bodies was strongly positive in a 1:1 dilution of serum and urine sugar was 4+. He had had diabetes mellitus for 12 years and had been hospitalized here eight times previously for ketoacidosis. On one prior admission, an ulcer on the right great toe necessitated amputation of the affected toe. The clinical impressions of the attending physician were diabetes mellitus, neuropathy, chronic pyelonephritis with possible acute exacerbation, and ketoacidosis precipitated by a combination of mismatching of insulin with diet and injudicious

intake of alcoholic beverages. Therapy on admission was 170 units regular insulin. At 5:00 p.m. on the day of admission, blood glucose was 321 mg. per 100 ml., urine sugar was 3+, and the test for ketones still strongly positive. At 9:00 p.m. on the same day, urine sugar was 1+ and the test for ketone bodies showed a trace in undiluted serum. At 1:00 a.m. on December 29, urine sugar was negative, test for ketones (serum) was negative, CO_2 capacity of serum was 26.7 mMoles per L., and blood sugar was 357 mg. per 100 ml. On January 3, 1961, these values were 31.4 and 344, respectively. He was discharged on 45 units NPH insulin each day.

Discussion. The data presented appear to indicate that normal human serum inhibits the growth of *R. oryzae* in some manner other than through classical antibody mechanisms. In support of this contention are the following: (a) complement could not be involved due to the persistence of inhibition after heating serum to 56° C. for 2 hours; (b) phagocytosis was eliminated as a possible mechanism through the use of serum which was always frozen before use in assays; (c) a partially purified globulin fraction from normal serum was not inhibitory; (d) dialysis removed the inhibitory factors; (e) removal of properdin from serum resulted in no alteration of the inhibitory titer; (f) agglutinating antibody apparently does not alter the viability of microorganisms (Raffel[10]); thus, an agglutinating antibody would not have been expected to result in inhibition of growth in these experiments. Likewise, a substance of a similar nature to the ablastin described by Taliaferro[13] seems to be excluded since this agent was associated with the globulin fraction in his experiments and its activity could be demonstrated after fractionation.

The data also indicate that the factors responsible for inhibition of *R. oryzae* are different from the antidermatophyte and anti-candida complexes studied by Roth *et al.*[11]. The former was dialyzable but heat labile; the latter resisted heating to 56° C. for 1 hour but could not be demonstrated in umbilical cord serum. Those authors also failed to report any initial stimulation of growth at low serum concentration prior to inhibition at higher concentrations.

The results of heat treatment and dialysis suggest a single substance or a number of very closely related substances as being responsible for the inhibition demonstrated. In view of the average 24 Å radius of the dialysis sack pores, some idea of the size of the molecules can be envisaged. Bond lengths between carbon and other elements are of the magnitude of 1.1 to 2.3 Å, while carbon to carbon bonds range in length from approximately 1.2 to 1.5 Å. The active substance or substances would thus appear to be of relatively low molecular weight.

The fact that a marked departure from normal serum response curve was demonstrated with sera from 2 of 6 patients with diabetes mellitus, a disease known to predispose to mucormycosis, may be of some significance; but extreme caution must be employed in interpretation until a larger series is examined. Unfortunately, a thorough history could not be obtained in regard to one of these patients, but the other was in known acidosis on admission and the inhibitory titer of his serum paralleled to some extent recovery from acidosis. Schofield and Baker[12] have shown that mice are most susceptible to experimental mucormycosis during the acute acidotic stage of alloxan-induced diabetes mellitus.

The apparent change in inhibitory titer during an episode of acidosis and its return to normal after control of the acidosis, if confirmed in other patients, raises a number of interesting questions. Primary among these would seem to be whether loss of inhibitory

capacity from the serum is a result of the actual absence of a specific inhibitor, or the presence of a substance or substances which stimulate growth of the fungus to such an extent that the depressing effects of the inhibitor are overcome.

Attempts are now underway to characterize further the factor or factors responsible for inhibition.

Summary. Inhibition of the growth of *Rhizopus oryzae* by normal human serum was demonstrated. Inhibition was apparently produced through other than classical antibody mechanisms. The factors responsible for inhibition are apparently different from the anti-dermatophyte and anti-Candida complexes described by other authors. Serum from 2 of 6 patients with a disease known to predispose to mucormycosis had a markedly reduced ability to inhibit the test organism.

ADDENDUM: After this report had been accepted for publication, it was noted that much better control of pH in the culture flasks could be obtained by the use of tris buffer (tris [hydroxymethyl] aminomethane) at a final concentration of 0.1 M and an initial pH of 7.0 or 7.3, than with the phosphate buffer employed. The low (0.005 M) phosphate concentration used was necessitated by the formation of a precipitate with Sabouraud medium when the former was used in higher concentrations. No such precipitate was noted with the tris buffer which has the added advantage of being physiologically and pharmacologically inert.

ACKNOWLEDGMENTS: 1. The authors are grateful to the following individuals at the Durham Veterans Administration Hospital and Duke Medical Center who have facilitated collection of sera from several sources: Mrs. Elizabeth Pendergrass, Mr. H. W. Craig, Mrs. Agnes Graham, Mrs. M. H. Loos and Dr. Wirt Smith. Dr. Ernest Chick prepared the properdin-free serum.

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SUMMARIO IN INTERLINGUA

Studios de Fungos Opportunistic. I. Inhibition de *Rhizopus oryzae* per Sero Human

Esseva demonstrate le inhibition del crescentia de *Rhizopus oryzae* per normal sero human. Le inhibition esseva effectuate apparentemente per mechanismos altere que le mechanismos classic de anticorpore. Le factores responsabile pro le inhibition differeva apparentemente ab le complexos anti-dermatophytic e anti-candidic describite per altere autores. Sero ab 2 de 6 patientes con morbos que cognoscitemente predispone al disveloppamento de mucormycosis habeva un marcatemente reducite potentia de inhibir le organismo experimental.

GROWTH INHIBITION OF CRYPTOCOCCUS NEOFORMANS BY CELL FREE HUMAN SERUM

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Inhibitory influence upon growth of microörganisms by human blood, particularly the serum fraction, has been known for many years. This inhibitory effect is mediated through specific antibodies (agglutinins, precipitins, and others) present in the serum and has also been found to be present in the absence of specific antibodies. It is this latter characteristic which is the basis for the present study.

Antibacterial qualities of human serum have been noted for a long time, even in the absence of specific antibodies. Jacox[7] noted that Nuttal first reported such an effect in 1888. In the same paper[7], a substance in the serum was noted to be bactericidal for *Bacillus subtilis, Bacillus anthracis* and *Streptococcus hemolyticus*. Subsequent studies (Hirsch[4,5], Myrvik and Weiser[13]) have shown that extracts of cells, particularly leukocytes from rabbits, have antibacterial effects against *Bacillus subtilis*. Other studies (Ekstedt[2], Kochan and Raffel[9], Wood, Ono and Bessey[18,19]) have indicated the presence of nonspecific, noncellular inhibiting factors in serum. A difference in this effect has been noted between plasma serum and blood serum and is apparently related to the platelets (Hirsch[6]). Some of this activity was found to be independent of complement. The presence of this activity in the newborn has been documented (Dancis and Kunz[1]).

A system of nonspecific bacterial inhibition has been clarified to some extent by the discovery and characterization of the properdin system (Lepow *et al.*[10], Pillemer *et al.*[15], Wardlaw and Pillemer[17]). This system was shown to be nonspecific, to require magnesium ions and the four components of complement, to be labile at 56° C. for one half hour, and to be bactericidal for many strains of bacteria.

Though considerable work has been done relative to the antibacterial effects of cell free serum, little has been published concerning antifungal activity. The present study deals with this aspect of nonspecific immunity in relation to the fungus *Cryptococcus neoformans*.

Materials and Methods. The strain of Cryptococcus used throughout this study was a fresh isolate from an active human case of cryptococcosis and was obtained at postmortem examination. It was grown with ease in pure culture and was maintained by repeated subcultures on Sabouraud's dextrose agar. It is totally inhibited in growth by .01 μg. per ml. of amphotericin B, is pathogenic for mice and grows equally well at 20° C. and 37° C.

Serum specimens were obtained from 7 patients and 3 normal men, the diagnoses of whom are listed in Table 1. The sera were frozen as soon as separated from the cells and not thawed until tested. The following

systems were set up for each serum sample studied: One set of 7 serology tubes, each containing 2.25 ml. of human serum and 2.25 ml. of Sabouraud's dextrose broth, was paired with 7 tubes containing only 4.5 ml. of Sabouraud's dextrose broth. To the first tube of both broth and broth plus serum systems, was added 0.5 ml. of a saline suspension of *Cryptococcus neoformans* (characterized above) containing 50 million cells per ml. After thorough mixing in the tube (concentration 5 million cells per ml.), 0.5 ml. of each first tube was transferred to each second tube, thoroughly mixed, and the transfer carried out through the 7 tubes in each system. At the same time, 0.1 ml. of the yeast cell suspension was placed in each of 2 perfume bottles containing Sabouraud's agar to check the viability of the cells used. The resulting concentrations of organisms in both the broth and the broth plus serum systems were 5 million cells per ml. by 10-fold dilution to 5 cells per ml. (It is recognized that because of the mucoid capsule, good distribution of Cryptococcus yeast cells is difficult to obtain; therefore, great care was exercised in mixing each tube thoroughly before the 0.5 ml. aliquot was transferred to the next tube.)

The system thus set up was allowed to incubate at 37° C. for 10 days. At the end of that time, pour-plates of Sabouraud's dextrose agar (containing 1000 units of penicillin and 2000 units of streptomycin per ml. of media) were made using 0.5 ml. from each tube of both the broth and the broth plus serum. These plates were incubated at 20° C. and were examined for growth at 4 days, 7 days and 15 days. All colonies were counted where possible, but, where coalescent growth occurred, "heavy" was recorded rather than the actual number of colonies.

Results. The results of this study are seen in Fig. 1. The viability control plates supported heavy growth within 3 to 4 days despite the small number of cells in the last 2 tubes. Since the experiments were run on only 2 or 3 different patients' sera at one time, the control observation was made at least three times during these experiments, because a control was set up with each run.

TABLE 1.—DIAGNOSES OF PATIENT:SOURCES OF SERA

L.P.	Normal Control
W.D.	" "
D.F.	" "
R.V.	Arteriosclerosis, ulcers of legs, right upper lobe infiltrate, etiology unknown
P.B.	Emphysema, arteriosclerotic heart disease, pyelonephritis
C.R.	Cholecystitis with mild diabetes and hypertension, arteriosclerosis and myocardial infarct
R.J.	Carcinoma of thyroid
B.C.	Myeloblastic leukemia
C.S.	Hodgkin's disease with terminal cryptococcosis
J.K.	Malignant lymphoma

As can be seen in Fig. 1, every serum sample inhibited growth completely in at least one tube. Two serum samples (R.J. and B.C.) inhibited growth in all but the tubes containing 5,000,000 cells per ml. In all tubes, the change from heavy growth to complete inhibition was gradual indicating a quantitative relationship between the concentration of inhibiting substance and the number of cells present. This might suggest a "salting out" effect of the inhibitory substance by the yeast cells present.

Discussion. The results of this study indicate that a definite inhibition of growth of *Cryptococcus neoformans* is

produced by human serum from a variety of patients and normal individuals. These studies have been extended and the effect has been consistent for all sera tested.

Since the possibility existed that some drug administered to the patients in this series was responsible for the serum inhibitory effect, 3 physicians were included in the study and are represented by the first three entries on Fig. 1. Despite the absence of any drug therapy, these three serum samples were as effective as the 7 patients' samples. The charts of the 7 patients were screened and no orders for common drugs were found.

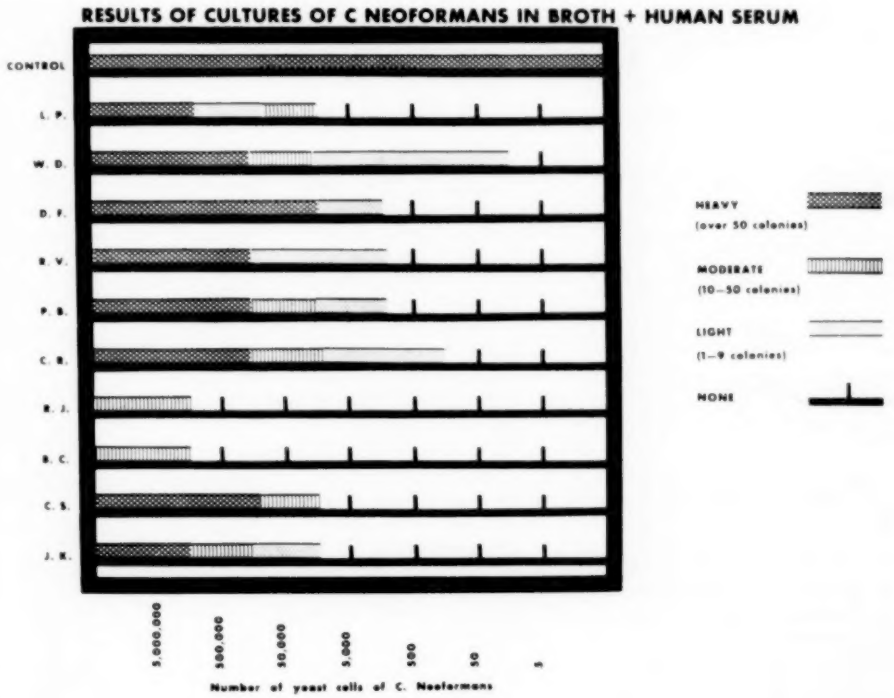

Fig. 1

Cryptococcus neoformans is an organism widely found in nature, but the disease cryptococcosis is quite uncommon. Since many animals and men are exposed, it would appear that variation in resistance of humans or variation in pathogenicity of the strains of *C. neoformans*, or both must explain the relative rarity of cryptococcosis. Most strains isolated from nature by Emmons[3] and Kao[8] were pathogenic for animals and were characterized as essentially similar to strains isolated from human cases. Therefore, variation in pathogenicity does not seem to play an important role although this factor cannot be dismissed with certainty.

Human resistance to infection with *Cryptococcus neoformans* seems to vary considerably since many cases of cryptococcosis occur in patients with lymphomatous disease of some type (Zimmerman[20], Zimmerman and Rap-

paport[21]). Just what the defect in resistance in these patients is, is not known. However, the fact that they are less resistant to many infections is a well-documented clinical observation (Lewis, Hopper and Scott[11], Murray and Brandt[12], Nelson, Goodman and Oster[14], Rodger, Terry and Binford[16], Zimmerman[20], Zimmerman and Rappaport[21]).

Previous studies have shown the existence of bacteriostatic and bactericidal properties of a relatively nonspecific nature in human serum. The results of the present study would indicate that similar factors occur for at least one of the pathogenic fungi; namely, *Cryptococcus neoformans*. Since the suggestion was made that this factor was complement, inactivation of serum by heating to 56° C. for one-half hour was done in simultaneous systems with non-inactivated serum in 4 instances. No significant quantitative difference was observed in the Cryptococcus inhibition effect of inactivated and non-inactivated serum in 3 cases while inactivated serum was decidedly less effective in one case. Therefore, it can be said that this effect was not due to or dependent upon complement in at least 3 of the 4 cases.

The exact definition of what substance (protein, combinations of proteins, other substance) in the serum has produced this effect has not yet been determined but will be the subject of future studies. In addition, the effects of serum on other pathogenic fungi will be tested.

Summary. 1) The inhibition of growth of *Cryptococcus neoformans* by human serum from 7 patients and 3 normal men is reported.

2) The persistence of this effect even after heat inactivation of serum indicates that complement is not necessary for its occurrence.

3) Immunologic implications are discussed.

ACKNOWLEDGMENT: The authors wish to thank Dr. Jan Schwarz for helpful criticism and procedural corroboration.

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(Interlingua on page 624)

THE RELATIONSHIP BETWEEN PROTEINURIA AND FATTY ELEMENTS IN THE URINE SEDIMENT

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"The doubly-refractile lipoids present in the renal tissue can appear in the urine. Their presence in the urinary sediment along with other findings is a valuable adjunct in the diagnosis of various renal diseases. . . . . The lipoids appear in the urine either as single droplets or as clusters ('small fat balls') or in fresh urine mostly as clear fatty casts."

The above observations made by Munk in 1918[9] represent the original description of "oval fat bodies" and a discussion of their importance. Since then there have been several reports concerning the significance of these urinary sediment elements (Addis[1], Brice[2], Krupp[5], Lippman[6], Miloslavich[8], Quinn and Zimmerman[10], Rifkin *et al.*[11], Walz and James[12]). Most authors agree that oval fat bodies, which are desquamated tubular epithelial cells with marked fatty degeneration, are usually found in patients with renal parenchymatous disease. In a recent study, Walz and James[12] reported the presence of doubly refractile bodies in patients with both renal disease and systemic disease.

In 1954 (Quinn and Zimmerman[10]), an attempt was made to classify the droplet-containing epithelial cells of the urine sediment into three types. Epithelial cells with only a few droplets were called "type I," the typical oval fat bodies loaded with droplets were called "type III," while "type II" cells were considered to be an intermediate form. It was concluded that only true oval fat bodies were significant, since type I and type II cells were observed in various extrarenal conditions (Quinn and Zimmerman[10]).

The presence of oval fat bodies is considered particularly characteristic of massively proteinuric disease (Addis[1], Brice[2], Lippman[6], Miloslavich[8], Munk[9], Quinn and Zimmerman[10]). Since it has been suggested that proteinuria may lead to (Addis[1], Merrill[7]) or depend on tubular abnormality (Freeman and Jockes[3]), it seemed of interest to attempt to correlate the degree of proteinuria with the number of oval fat bodies in the urine. To our knowledge this has not been attempted by others. Previous studies (Brice[2], Quinn and Zimmerman[10], Rifkin *et al.*[11], Walz and James[12]) on the significance of oval fat bodies have been carried out with randomly selected patients with and without proteinuria. This project was limited to patients with proteinuria, regardless of its cause.

Method. Urines from 100 consecutively available patients with proteinuria were examined (Table 1). In many of these patients repeated examinations of the sediments were performed, but only the first urine examined was included in the statistical evaluation. The specimens were collected from 7:00 p.m. to 7:00 a.m. in a bottle containing 0.5 ml. of 10% formaldehyde. The fatty elements were found to be well preserved up to 12 hours following the collection of the specimens. The volume of the urine was measured and the urinary protein content was quantitatively determined by the Kingsbury-Clark method[4]. A quantity of urine representing the output of 1/5 of an hour was then centrifuged for 5 minutes in a 15 ml. calibrated tube. The supernatant was discarded leaving 0.5 ml. of the centrifuged urine; 3 drops of Sudan III dye were then added to the agitated sediment. The sediments were examined microscopically after 10 minutes of staining. In screening the sediments for the presence of fatty elements, the "hanging drop" technique was found to be useful. For this a slide with a concavity in the center such as is used in bacteriologic and hematologic procedures, was used. A small drop of stained urine was placed on a cover slip and a small amount of vaseline was applied along its edges. The slide was then placed with its concavity over the drop and inverted. By using the fine adjustment, the stained fatty elements became readily visible at various levels of the hanging drop of urine. This method of screening for fatty elements was found preferable to the usual technique. When fatty elements were present, a Spencer-Bright Line (improved Neubauer method) Hemocytometer was used for semi-quantitative estimation of the fat-containing epithelial cells. The cells were counted within an area in the counting chamber, as done in the Addis count technique utilizing six large squares[1].

Results. The conditions with proteinuria encountered in the 100 consecutive patients studied are listed in Table 1. The largest number of fatty elements were present in the patients with various types of nephrotic syndrome.

Fatty elements (type I and type II cells, oval fat bodies, and fatty casts) were found in 39% of the 100 urines examined. Type I cells were found in 34%, type II in 32%, oval fat bodies in 25% and fatty casts in 18% of the patients.

There was a positive correlation between the total number of fatty elements and the degree of proteinuria (Fig. 1). When the proteinuria exceeded 4.0 gm. per 12 hours, one or more of the various fatty elements were always present (Fig. 1). Figure 2 represents the number of oval fat bodies plotted against the amount of proteinuria.

Most of the oval fat bodies were seen in patients with a blood urea nitrogen (BUN) below 30 and were not seen with blood urea nitrogen determinations above 50 mg. per 100 ml. (Fig. 3). Inspection of Fig. 4 reveals an apparent inverse relationship between the level of BUN and degree of proteinuria.

Discussion. The oval fat body is an element which, when viewed with the low power, appears as a dark, almost black body. When viewed under high power the glistening, refractile nature of the individual component globules becomes apparent. It is not difficult, with some experience, to recognize an oval fat body by ordinary microscopy technique. For further identification one may resort to the use of polarized light or specific fat stains such as Sudan III, Sudan IV, or Oil Red O dye which stain the majority of fat globules bright red or orange-red.

In this study, oval fat bodies were seen most frequently in patients with nephrotic syndromes due to various causes. These cells also were seen in patients with toxemia of pregnancy, diabetes mellitus (even without the Kimmelstiel-Wilson syndrome) and cardiovascular diseases, such as organic heart disease and cerebrovascular accidents. As has been shown before, it appears that the demonstration of oval fat bodies is not limited to patients with intrinsic renal disease. On the

TABLE 1.—SUMMARY OF RESULTS

TABLE 1.—SUMMARY OF RESULTS

Disease	*Number of Patients Studied*	*Number of Sediments Studied*	*Urinary Protein gm./12 Hours*	*Number of Total Fatty Elements per Sediment*	*Number of "Type I Cells" per Sediment*	*Number of "Type II Cells" per Sediment*	*Number of "Oval Fat Bodies" per Sediment*	*Number of Fatty Casts per Sediment*
Renal								
Acute Glomerulonephritis	2	4	1.03	2.5	2.0	0.0	0.5	0.0
Membranous Glomerulonephritis	2	31	3.13	18.5	3.8	6.6	5.4	2.7
Lipoid Nephrosis	3	11	1.46	10.7	3.2	3.2	3.0	1.3
Acute Pyelonephritis	1	1	0.15	0.0	0.0	0.0	0.0	0.0
Chronic Pyelonephritis	8	11	0.11	0.3	1.5	1.0	0.0	0.0
Renal Vein Thrombosis	1	1	1.00	10.0	4.0	3.0	3.0	0.0
Renal Calculi	2	2	0.32	0.0	0.0	0.0	0.0	0.0
Hepato-Renal Syndrome	1	1	0.71	0.0	0.0	0.0	0.0	0.0
Kimmelstiel-Wilson Syndrome	1	3	1.09	12.0	1.0	1.5	3.0	6.5
Diabetic Nephropathy	1	2	0.55	5.5	1.5	2.5	1.5	0.0
Toxemia of Pregnancy	8	13	0.81	9.2	2.7	3.4	2.1	1.0
Cardiovascular								
Coronary Heart Disease								
With Failure	3	3	0.03	3.0	1.5	2.5	1.5	0.5
Without Failure	12	13	0.17	0.8	0.3	0.3	0.2	0.0
Luetic Heart Disease								
With Failure	1	1	0.06	2.0	2.0	0.0	0.0	0.0
Rheumatic Heart Disease								
With Failure	2	2	0.01	0.0	0.0	0.0	0.0	0.0
Without Failure	2	2	0.04	1.0	0.5	0.5	0.0	0.0
Hypertensive Heart Disease								
With Failure	2	2	1.15	0.0	0.0	0.0	0.0	0.0
Cerebrovascular "Accident"	3	3	0.07	1.0	0.3	0.3	0.3	0.0
Neoplastic								
Carcinoma	3	3	0.21	0.0	0.0	0.0	0.0	0.0
Leukemia	1	1	0.34	0.0	0.0	0.0	0.0	0.0
Others								
Pneumonia	5	5	0.13	1.0	0.6	0.4	0.0	0.0
Appendicitis	1	1	0.54	0.0	0.0	0.0	0.0	0.0
Diabetes*	11	12	0.24	0.4	0.3	0.1	0.1	0.0
Miscellaneous	24	28	0.24	0.6	0.3	0.3	0.1	0.0

*None with apparent renal involvement.

other hand, from previous observations (Quinn and Zimmerman[10], Rifkin *et al.*[11]), and from this study, it appears that oval fat bodies are rarely seen in some types of renal disease, such as nephrosclerosis, pyelonephritis, acute glomerulonephritis, cystic disease of the kidneys, renal carcinoma or those diseases producing acute renal failure.

The greatest incidence of oval fat bodies is in patients with massive proteinuria as in the nephrotic syndrome. Although we studied only patients with proteinuria, it is of interest that oval fat bodies may, on rare occasions, be seen in patients without proteinuria. Quinn and Zimmerman[10] reported 3 patients with oval fat bodies who had

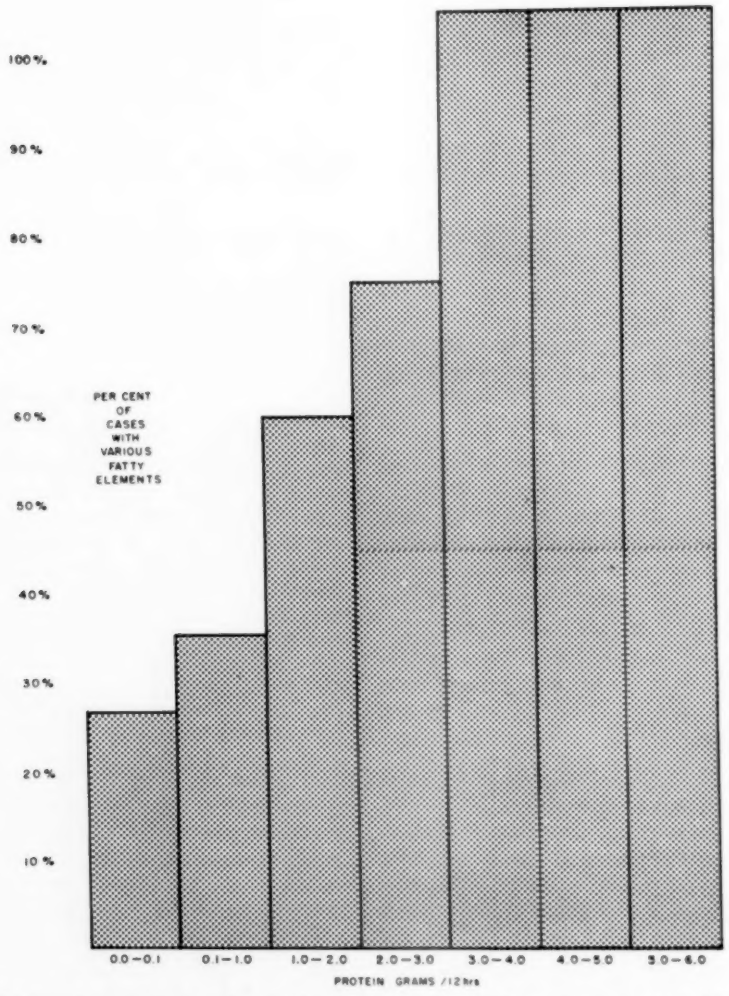

Fig. 1.—Bar graph representing the number of patients with fatty elements plotted against the amount of urinary protein present. As can be seen, the number of patients with fatty elements in their urine increases as the urinary protein increases. Above 4.0 gm. of urinary protein all the patients had one or more of the fatty elements present in their urine.

no proteinuria at the time of sediment study. These patients had heart disease with congestive failure. We have studied 2 children with lipoid nephrosis, who, while receiving prednisone, did not have proteinuria; but small numbers of oval fat bodies were seen in several of their urine specimens early molded by the tubular lumen into the shape of a cylinder. The incidence of fatty casts in this study was lower than that of oval fat bodies. The presence of fatty casts did not correlate as well with proteinuria as did the oval fat bodies. On the other hand, it is of interest that fatty casts were seen al-

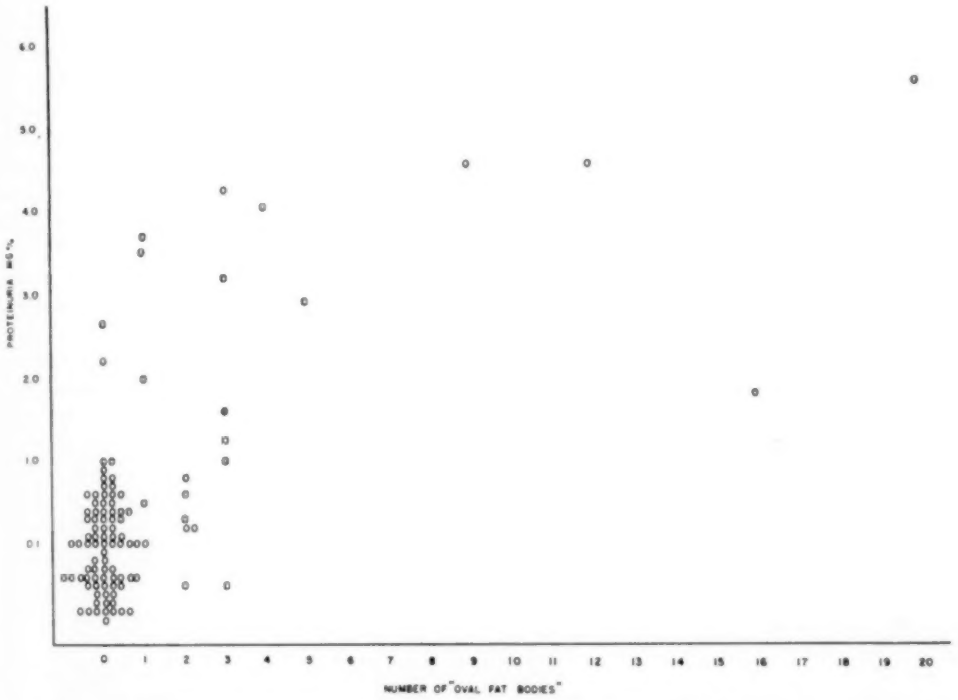

Fig. 2.—Scattergram representing the number of oval fat bodies plotted against the amount of proteinuria. It is apparent that the number of oval fat bodies increases as the amount of proteinuria increases, although the presence of proteinuria was not always associated with the presence of oval fat bodies.

in the course of the prednisone therapy. Because of the lack of proteinuria, these 2 patients were not included in this study. It is possible that tubular epithelial cells which had undergone fatty degeneration were still being desquamated in this early stage of therapy.

Fatty casts are probably formed by a conglomeration of oval fat bodies most exclusively in patients with intrinsic renal disease.

The remaining fatty elements type I and type II cells were seen in patients with both renal and systemic diseases. This has been described previously (Quinn and Zimmerman[10]). It, therefore, appears that type I and type II cells are the least specific of the fatty elements. Nevertheless, a good corre-

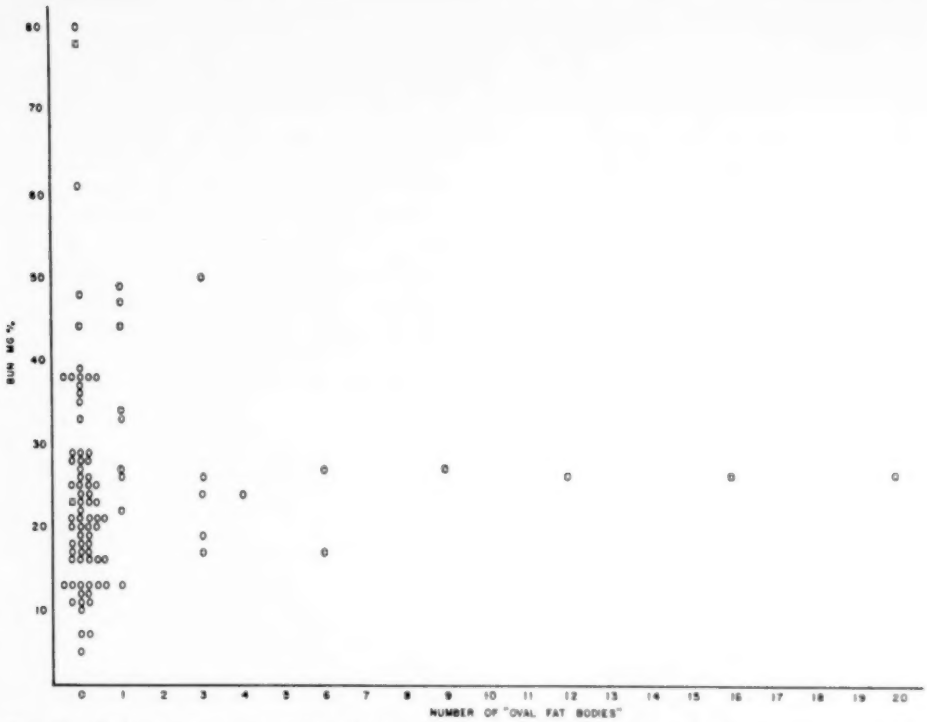

Fig. 3.—Scattergram representing the number of oval fat bodies plotted against the BUN level. Most oval fat bodies were present in the urines of patients with a BUN level below 30 mg. per 100 ml. and none was seen above 50 mg. per 100 ml.

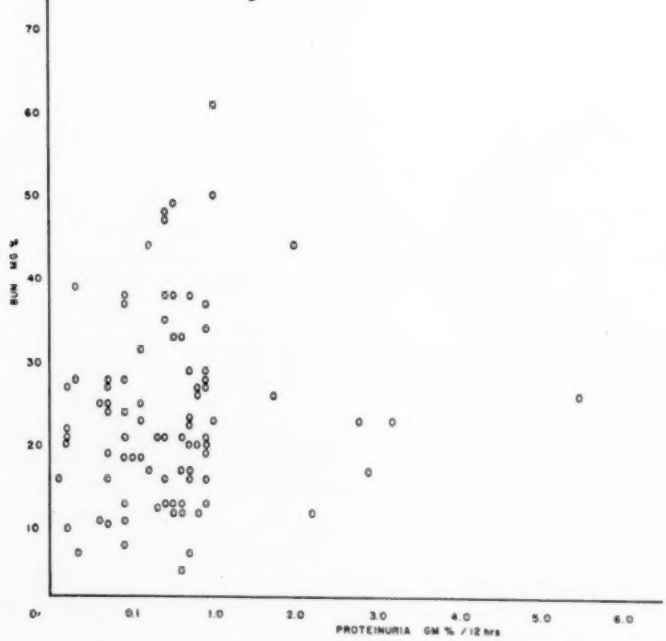

Fig. 4.—Scattergram representing the amount of proteinuria plotted against the level of BUN. Proteinuria appeared to decrease as the BUN level increased.

lation between the presence of these cells and proteinuria was observed.

It has been suggested (Freeman and Jockes[3]) that proteinuria results, at least in part, from tubular abnormality. Others (Addis[1], Merrill[7]) have suggested that the large amounts of protein produce changes, leading to the eventual oval fat body. The positive correlation between the degree of proteinuria and number of fatty elements in the urine sediment accordingly is to be expected.

There does not appear to be close correlation between the BUN level and the number of oval fat bodies in the urine. Nevertheless, at levels of azotemia above 50 mg. per 100 ml., oval fat bodies are not found in the urine. At this level of azotemia, proteinuria also was slight or absent. The decrease in proteinuria in patients undergoing transition from the nephrotic to the terminal stage of nephritis has been well-discussed by Addis[1]. The decrease in glomerular perfusion and filtration in the patients with chronic renal insufficiency results in decreased protein in the glomerular filtrate and, accordingly, in the urine. The absence of oval fat bodies from the urine in a stage of renal disease characterized by only minimal proteinuria is consistent with the concept discussed above that tubular degeneration results from proteinuria.

Summary. 1. Urinary sediments from 100 patients with proteinuria were examined for the presence of fatty elements. The largest number of these were present in the patients with various types of nephrotic syndrome.

2. Epithelial cells with only a few droplets of fat were classified as type I, the typical oval fat bodies loaded with droplets were called type III, while type II cells were considered to be an intermediate form.

3. Fatty elements (type I and type II cells, oval fat bodies and fatty casts) were found in 39% of the 100 urines examined. Type I cells were found in 34%, type II in 32%, oval fat bodies in 25% and fatty casts in 18% of the patients.

4. When the proteinuria exceeded 4.0 gm. per 12 hours, one or more of the various fatty elements were always present, although there was a positive correlation between the total number of fatty elements and the degree of proteinuria.

5. From the results of this study it has been inferred that tubular degeneration may result from proteinuria.

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SUMMARIO IN INTERLINGUA

Le Relation Inter Proteinuria e le Presentia de Elementos Grassiose in le Sedimento del Urina

1. Le sedimentos del urina de 100 patientes con proteinuria esseva examinate pro le presentia de elementos grassiose. Le plus grande numero de tales esseva trovate in le casos de patientes con varie typos de syndrome nephrotic.

2. Cellulas epithelial con solmente pauc guttettas de grassia esseva classificate como typo I, le characteristic corpores grasse oval cargate con guttettas esseva designate como typo III, e cellulas del typo II esseva illos que poteva esser considerate como un forma intermediari.

3. Elementos grassiose—*i.e.* cellulas de typo I e de typo II, corpores grassiose oval, e cylindros grassiose—esseva trovate in 39 del 100 urinas examinate. Cellulas de typo I esseva presente in 34, cellulas de typo II in 32, corpores grassiose oval in 25, e cylindros grassiose in 18.

4. Quando le proteinuria excedeva 4,0 g per 12 horas, un o plures del varie elementos grassiose esseva presente sin exception. Il existeva un correlation positive inter le numero total de elementos grassiose e le grado de proteinuria.

5. Super le base del resultatos del presente studio il esseva concludite que degeneration tubular pote resultar ab proteinuria.

SUMMARIO IN INTERLINGUA

(See page 613 for original article)

Inhibition del Crescentia de Cryptococcus neoformans per Acellulate Sero Human

1. Es reportate le inhibition del crescentia de *Cryptococcus neoformans* per sero human ab 7 patientes e 3 masculos normal.

2. Le persistentia de iste effecto mesmo post thermo-inactivation del sero indica que complemento non es necessari pro su occurrentia.

3. Corollarios immunologic del constatation es discutite.

INCIDENCE OF VARIOUS COAGULATION DEFECTS AND THEIR ASSOCIATION WITH DIFFERENT DISEASES*

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From July 1, 1954, to June 30, 1960, 3,342 patients were studied in the Coagulation Laboratory of the Hospital of the University of Pennsylvania. Four thousand six hundred seventy-two (4,672) "coagulograms" were done on these patients. Some of the results from this laboratory have been reported (McCreary and Wurzel[7], Wurzel[14,15], Wurzel, Kirby and Molthan[16]). Most of the patients were referred for study because they were bleeding or had a history of bleeding. Other patients were referred for evaluation of the coagulation mechanism prior to certain operative procedures, and a few patients were studied to see if a coagulation defect was present as part of another disease process.

Methods: A "coagulogram" refers to the coagulation tests routinely performed by standard methods (Quick[10], Stefanini and Dameshek[11]) with modifications in this laboratory and includes the following: Lee-White clotting time, prothrombin consumption test, platelet count, recalcification time, clot retraction, one-stage prothrombin test, and measurement of Factors V and VII.

Factor II (prothrombin) was measured by the 2-stage prothrombin test (Warner, Brinkhous and Smith[13]) as modified by Ware and Seegers[12] when indicated.

On the blood of all patients who had tests or histories compatible with a diagnosis of hemophilia, thromboplastin generation tests were done by the method of Biggs and Douglas[1].

Fibrinogen concentrations were not routinely measured, but when needed Fibrindex† was used or a chemical determination was made (Cameron, Guthrie and White[2]). Other tests were devised as required by the history and findings of any given patient.

Results. One thousand one hundred-twelve (1,112) patients (30.3%) had normal coagulograms.

THROMBOCYTOPENIA. As was to be expected, thrombocytopenia was the most common abnormality found (Quick[10]). Eight hundred sixty-seven (867) patients (26%) had platelet counts below 150,000 per c.mm. Sixty-five (65) of these patients had idiopathic thrombocytopenic purpura. Of this group 60 had counts below 100,000 per c.mm.

It is of great interest that 74 patients with counts between 100,000 and 150,000 and 15 patients with platelet counts below 100,000 had either rheumatic heart disease or congenital heart disease.

Two patients with hereditary hemorrhagic telangiectasia had thrombocytopenia, one below 100,000. The other patient also had long standing uremia. During the course of this study, 4 patients with thrombotic thrombocytopenic purpura were seen. These patients are being reported in detail separately (Lawler, Barrows and Frost[6]). Tables 1 and 2 give a

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†Ortho Pharmaceutical Corp., Raritan, N.J.

TABLE 1.—CONDITIONS ASSOCIATED WITH PLATELET COUNTS BELOW 100,000 PER C.MM.

Disease or Conditions	*No. of Patients*
Idiopathic thrombocytopenic purpura	60
Uremia	49
Postoperative open heart operation	35
Malignant tumors	27
After massive blood transfusions	22
Gastrointestinal tract bleeding	20
Cirrhosis of the liver	15
Acute leukemia	14
Postoperative	14
Chronic lymphatic leukemia	14
Aplastic anemia	14
Various medications	12
Infections	10
Disseminated lupus erythematosus	10
Hypersplenism	10
Rheumatic heart disease	9
Congenital heart disease	6
Chronic myelogenous leukemia	5
Myelofibrosis of the bone marrow	4
Polycythemia vera	4
Thrombotic thrombocytopenic purpura	4
Lymphosarcoma	4
Hodgkin's disease	4
Unknown diagnosis	4
Multiple myeloma	3
Each of the following	2
Premature separation of the placenta, erythroleukemia, eclampsia, Gaucher's disease, post-intravenous fat, thromboses, aortic aneurysm, sarcoidosis.	
Each of the following	1
Fat embolus, tuberculosis, hereditary hemorrhagic telangiectasia, premature infant, congenital hemolytic anemia, benign pericarditis, postoperative hypothermia, septic abortion, cerebral vascular accident, undiagnosed collagen disease, biliary cirrhosis, pernicious anemia.	

TABLE 2.—CONDITIONS ASSOCIATED WITH PLATELET COUNTS OF 100,000 TO 150,000 PER C.MM.

Disease or Conditions	*No. of Patients*
Uremia	59
Postoperative open heart operation	59
Rheumatic heart disease	52
Gastrointestinal tract bleeding	32
Postoperative	26
Unknown diagnosis	26
Malignant tumors	25
Congenital heart disease	22
Various medications	16
Post-massive transfusions	12
Menorrhagia	10
Cerebral vascular accidents	9
Cirrhosis of the liver	9
Polycythemia vera	7
Pre-operative	6
Non-thrombocytopenic purpura	5
Idiopathic thrombocytopenic purpura	5
Lymphosarcoma	5
Chronic lymphatic leukemia	5
Bacterial infections	5
Hypertension	5
Hypersplenism	4
Epistaxis	4
Thrombosis	4
Chronic myelogenous leukemia	4
Tuberculosis	3
Eclampsia	3
Biliary cirrhosis	3
Genitourinary bleeding	3
Each of the following:	2
Multiple myeloma, aortic aneurysm, normal pregnancy, hemachromatosis, incomplete abortion, sickle cell anemia, benign pericarditis, sarcoidosis.	
Each of the following:	1
Gout, retinal hemorrhage, post-trauma, premature separation of the placenta, postpartum, cystic fibrosis, uremia with hereditary hemorrhagic telangiectasia, hematoma, aplastic anemia, sickle cell-C disease, premature infant, myeloproliferative disease, disseminated lupus erythematosus, Hodgkin's disease, reticulum cell sarcoma, measles, arteriovenous fistula, hemangioma, rheumatoid arthritis, hypogammaglobulinemia, diabetes mellitus and disseminated lupus erythematosus with circulating anticoagulant.	

complete list of the conditions in which thrombocytopenia was found.

NON-THROMBOCYTOPENIC PURPURA. Two hundred thirty-four (234) patients gave a history of purpura usually ecchymosis or ready bruising. Of these, 229 had no abnormalities in the "coagulogram." Five had platelet counts below 150,000 per c.mm., but usually in the 120,000 to 140,000 range. In this range it is unlikely that the purpura was caused by the lowered platelet count. No platelet counts below 100,000 were found in this group.

LIVER DISEASE. One hundred fifty-one (151) patients had cirrhosis of the liver. Of these, 21 had normal "coagulograms" and 130 had one or more abnormalities. Nine (9) had a platelet count between 100,000 and 150,000 per c.mm. and 15 had platelet counts below 100,000 as the only abnormality. The greatest number (63) had a combination of thrombocytopenia, decrease in Factor II (prothrombin) or Factor V concentrations, or both. In the latter group were 3 patients with Wilson's hepatolenticular disease. One patient had thrombocytosis (platelet count 968,000 per c.mm.) as the only abnormality.

HEMOPHILIA. Ninety-three (93) patients had hemophilia. Sixty-nine (69) (74.2%) had hemophilia A; 19 (20.4%) had hemophilia B; and 5 (5.4%) had hemophilia C. Only one of the patients with hemophilia had a circulating anticoagulant; he was a boy with hemophilia A. One of the patients with hemophilia A was a female and she will be reported in detail elsewhere (Mellman *et al.*[8]).

CIRCULATING ANTICOAGULANTS. Three other patients with naturally occurring anticoagulants were found. One of these had disseminated lupus erythematosus. Two had idiopathic anticoagulants, most likely antithromboplastins.

THROMBOCYTOSIS. Seventeen (17) patients with platelet counts over 700,000 per c.mm. were studied. Seven of these had polycythemia vera, 4 had chronic myelogenous leukemia and one each had acute tubular necrosis, carcinoma, and cirrhosis of the liver. Two patients were classified as having idiopathic thrombocytosis. The other patient's thrombocytosis occurred after a splenectomy.

ABNORMALITIES IN THE PROTHROMBIN COMPLEX AND AFIBRINOGENEMIA. One patient had a congenital deficiency of Factor V and one a congenital deficiency of Factor VII. Because of the comparative rarity of these defects, these patients will be described below. Three patients had a profound deficiency of Factor II (prothrombin) not related to anticoagulant therapy. Two of these had the malabsorption syndrome and one had a defect that was never defined even after prolonged study. Only one case of afibrinogenemia, not related to pregnancy or the postoperative period was found. This man had been bitten by a rattlesnake and has been reported (McCreary and Wurzel[7]).

Minor defects in the prothrombin complex and defects in the other coagulation tests not related to the present study will not be reported at this time.

Case Reports. CASE 1. CONGENITAL DEFICIENCY OF FACTOR V. J.S. at the time of study was an 18-year- old white male, who had a history of ecchymosis and ready bruising since birth. He was able to participate in strenuous exercise without serious complications. In fact, he played football on his high school team. During the football season, he noted more ecchymotic areas. He never had joint symptoms and had no other history of bleeding.

Coagulation studies revealed a prolonged recalcification time and prothrombin time, the latter due to a marked deficiency of Factor V (Table 3). The thromboplastin generation test was abnormal with his aluminum hydroxide adsorbed plasma, but normal when his serum was used in the reaction mixture. The defect in the prothrombin test could

be corrected by normal plasma and by plasma obtained from patients receiving Dicumarol therapeutically, but not by stored normal serum.

The only member of his family available for study was his father who had a normal "coagulogram."

The finding of an abnormal thromboplastin generation test does not necessitate making a diagnosis of hemophilia A, as Douglas[3] has shown that Factor V is needed for the production of throboplastin. O'Brien[9] found an abnormal thromboplastin generation test in his patient with Factor V deficiency. O'Brien's patient also had only a mild bleeding tendency.

CASE 2. CONGENITAL DEFICIENCY OF FACTOR VII. L.B., a white female, was first admitted to the Hospital of the University of Pennsylvania in June, 1948, at age 14, because of anemia secondary to menorrhagia. Extensive ecchymosis had been present all her life. There was no family history of a bleeding diathesis. She was the younger of 2 female children. At the time of her first admission, her prothrombin time was 10% of normal. The bleeding was controlled by fresh whole blood transfusion.

During the next 4 years many more hospitalizations were required to correct the recurrent anemia and to control the profuse uterine bleeding. In September, 1952, a hysterectomy was performed to prevent this recurrent need of blood.

Hemarthrosis also became a major problem in the clinical management of the patient in 1948.

TABLE 3.—COAGULATION RESULTS OF PATIENT WITH FACTOR V DEFICIENCY

		8/13/58	8/15/58
Clotting time (glass)		13′	
Clotting time (siliconized)			24′
Prothrombin consumption		37.2″	33.5″
Clot retraction		Good	Good
Platelet count		185,000	240,000
Recalcification time		450″	300″
Prothrombin time		35% (19.5″)	36% (19.3″)
Factor V		5%	0%
Factor VII		Normal	Normal
Two Stage Prothrombin (modified method)			98%
Thromboplastin Generation Test	Control	9.0 seconds (6 minute incubation)	
	Patient's serum	9.4 seconds (7 minute incubation)	
	Patient's adsorbed plasma	19.4 seconds (12 minute incubation)	
Prothrombin Time with dicumarolized (1:1)			12.5″*
with stored serum (9:1)			17.2″
with dicumarolized plasma (1:1)			14.5″†

*The normal plasma had a prothrombin time of 12.5″.

†The dicumarolized plasma had a prothrombin time of 29.2″.

Many "coagulograms" have been performed with similar results. (Prothrombin time over 60″ that could be corrected by stored serum.) The following coagulation tests were normal on multiple occasions: clotting time (in glass and in siliconized tubes), Russell's viper venom* time, recalcification time, platelet count, clot retraction, prothrombin consumption, two stage prothrombin tests, fibrinogen, and thromboplastin generation tests (with patient's serum or aluminum hydroxide adsorbed plasma, or both). There was no correction of the prothrombin time by barium sulfate adsorbed plasma or serum; or serum heated to 56° C. for 5 minutes.

*The Russell's viper venom was supplied by the Burroughs-Wellcome Co., Tuckahoe, N.Y.

TABLE 4.—EFFECT OF ADDITION OF AGED SERUM ON PROTHROMBIN TIME OF PATIENT WITH FACTOR VII DEFICIENCY

Patient	*L.B.*	*Mother*	*Father*	*Sister*	*Niece*	*Normal*
Prothrombin Time (seconds)	54.5	15.4	15.4	13.3	14.9	13.0
Correction by aged normal serum	12.6	14.5	15.0		12.0	

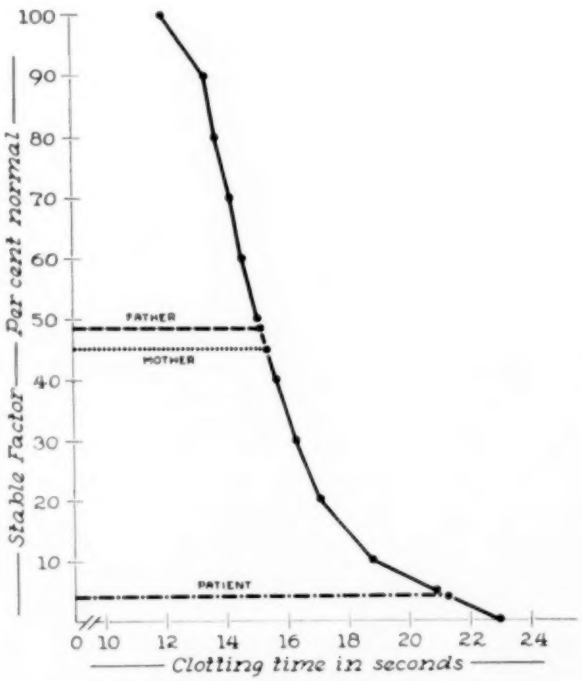

Fig. 1.—Employing a dicumarolized plasma with a prothrombin time of 23.8 seconds, the graph was prepared by using various dilutions of normal serums and determining the corrective effects of these on the prothrombin time. The corrective effects of the serum of the patient and her parents are also plotted to determine their % of Factor VII.

In June, 1956, 1 gm. of Vitamin K_1 (Mephyton)* was given by mouth for 8 consecutive days without appreciable change in the prothrombin time, though there was subjective improvement as far as the ecchymosis and joint manifestations were concerned. On August 2, and 3, 1956, 1 gm. of Vitamin K_1 (Mephyton) was given intravenously without change in the prothrombin time.

Only a small family group was available for study; the parents, one sister, and one niece. Table 4 shows the prothrombin time of these relatives. Only the sister had a normal prothrombin time. Only the niece's prothrombin time was fully corrected by serum. Slight correction of the parents' prothrombin time by serum was found. Figure 1 reveals that the patient had only 3% of the normal amount of Factor VII, while her father had 48.5% and her mother 45%.

*The Mephyton was supplied by Merck, Sharp and Dohme, West Point, Pa.

Discussion. The routine performance of a "coagulogram" has definite clinical value. It helps to differentiate bleeding due to a coagulation defect and bleeding due to other causes. It distinguishes between ecchymosis due to thrombocytopenic purpura and non-thrombocytopenic purpura. With the use of the thromboplastin generation test, the "coagulogram" distinguishes between the various kind of hemophilia.

Prior to surgical procedures, if abnormalities in the coagulation system are found, they can be treated intelligently. Fresh blood would be mandatory for a combination of thrombocytopenia and deficiency of Factor V but only fresh plasma, that can be stored in the blood bank in the frozen state, is needed if Factor V alone is decreased. To correct the defect in hemophilia A, fresh blood or plasma is needed but if hemophilia B or C is present the blood or plasma need not be fresh.

In many of the cases of thrombocytopenia associated with rheumatic and congenital heart disease, cardiac cirrhosis with secondary hypersplenism was probably present as an explanation in part of the thrombocytopenia. Many of these patients were also taking several drugs and some had platelet counts that became normal after the drugs were stopped.

The finding of 93 patients (2.8%) with hemophilia and only 65 (1.9%) with idiopathic thrombocytopenic purpura represents weighting of the figures in favor of hemophilia as this laboratory is used by many hospitals as a reference laboratory for studying patients with hemophilia. All patients admitted to the hospital with idiopathic thrombocytopenic purpura were not studied by this laboratory but all patients with hemophilia were studied. The frequency of the type of hemophilia is similar to other reported series (Jung[5]).

The combination of thrombocytopenia and deficiencies in the factors of the prothrombin complex as the most common defect found in cirrhosis of the liver is similar to the report of Finkbeiner *et al.*[4]. The latter group reported that platelets and Factor V were most often found deficient in patients with liver disease.

For most patients only a clotting time, recalcification time and platelet count are needed for the presumptive diagnosis of a first stage defect (hemophilia or thrombocytopenia). An abnormal Quick prothrombin test is usually adequate for presumptive diagnosis of a second stage defect. Prolongation of the clotting time, recalcification time and prothrombin time usually means a defect in the third stage of coagulation (hypofibrinogenemia). A positive tourniquet test in the presence of a normal platelet count usually signifies vascular or nonthrombocytopenic purpura. With these few tests most of the coagulation defects can be diagnosed. Only occasionally is a more complex study needed.

Summary. In a series of patients studied by means of a "coagulogram," thrombocytopenia was the most frequent abnormality found. The frequent association of thrombocytopenia with rheumatic and congenital heart disease was somewhat unexpected. The thrombocytopenia in some of these patients was probably due to cardiac cirrhosis of the liver with secondary hypersplenism, or to drug therapy.

Hemophilia was found more frequently (2.8%) than idiopathic thrombocytopenic purpura (1.9%) but this is accounted for by the fact that this laboratory serves as a reference laboratory for typing hemophilia.

Congenital deficiencies of Factor V and VII were rare in this series as only one patient with each defect was seen.

The incidence of congenital deficiencies of Factor I (fibrinogen), Factor II (prothrombin) or Factor X (Stuart factor) must be even less common as no patients with these diseases were found.

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SUMMARIO IN INTERLINGUA

Le Incidentia de Varie Defectos del Coagulation e Lor Association con Diverse Morbos

In un serie de patientes studiate per medio de un coagulogramma, thrombocytopenia esseva le anormalitate trovate le plus frequentemente. Le alte frequentia de un association de thrombocytopenia con morbo cardiac rheumatic e congenite esseva plus o minus inexpectate. In certes de iste patientes, le thrombocytopenia esseva probabilemente un effecto de cardiac cirrhosis del hepate con hypersplenismo secundari. In alteres, il pare justificate incriminar drogas therapeutic como factor etiologic in le disveloppamento del thrombocytopenia.

Hemophilia esseva trovate plus frequentemente que idiopathic purpura thrombocytopenic (2,8 e 1,9 pro cento, respectivemente), sed iste differentia se explica per le facto que nostre laboratorio servi como laboratoria de referentia in le typification de hemophilia.

Deficientias congenite de Factor V e VII esseva rar in iste series; solmente un patiente con le un e un con le altere defecto esseva incontrate. Le incidentia de congenite deficientias de Factor I (fibrinogeno), Factor II (prothrombina), e Factor X (factor Stuart) pare esser ancora plus basse; nulle casos de iste defectos esseva trovate.

THE COAGULANT PROPERTIES OF NORMAL AND ARTERIOSCLEROTIC AORTAS*

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Arteriosclerosis is the most common cause of intraarterial thrombosis. Since arteriosclerotic changes develop slowly and presumably may be present for years without the development of intraarterial thrombosis, the factors that precipitate thrombosis in arteriosclerotic vessels have been the subject of intensive study. Diminished blood flow, damage to the endothelial lining of vessels and increased coagulability of the blood are generally recognized as factors which predispose to intravascular thrombosis (Karsner[4]). Rupture or hemorrhage, or both, into arteriosclerotic plaques have also been considered important in precipitating thrombosis. Duguid has postulated that arteriosclerosis and intravascular coagulation are related and that intravascular coagulation is a normal process resulting in the deposition of fibrin on the endothelium which subsequently becomes incorporated into the intima, resulting in a characteristic atheroma[3]. According to this hypothesis, arteriosclerosis and subsequent thrombotic occlusion represent an extension of the normal process of intravascular coagulation. It is apparent, however, that the factors concerned with the initiation of thrombosis in arteriosclerotic vessels have not been clearly defined.

It has been shown that the blood vessels of man and animals possess thromboplastic activity (Astrup *et al.*[1], Campbell[2], Witte and Bressel[7]). Thromboplastic activity has been demonstrated in extracts of human and bovine aorta, primarily by the ability of such material to clot recalcified plasma. The demonstration of thromboplastic activity in the intima of vessels suggests a potential role in the development of intraarterial thrombosis. It seemed worthwhile to determine whether the development of arteriosclerosis altered the thromboplastic material found in normal vessels. There is ample reason for suspecting that the lipids which comprise the atheroma might have coagulant properties, since phospholipids are present in atheromas and the thromboplastic activity of platelets has been shown to be due to a phospholipid, phosphatidyl-serine (Troup *et al.*[6]). We have investigated the coagulant properties of normal and arteriosclerotic vessels by several techniques and compared their coagulant

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activity with human brain, a well recognized source of thromboplastic material. The results of these studies constitute the present report.

Material and Methods. The one-stage prothrombin time, prothrombin consumption and thromboplastin generation tests were performed according to methods described by MacFarlane and Biggs[5]. The blood vessels were obtained from necropsy material. A portion of thoracic aorta was removed and the vessels graded from 0 to 4+ depending upon the degree of atherosclerosis. A normal vessel (Grade 0) was considered to be one which showed a smooth endothelial lining without gross atheromatous plaques. Grade I vessels contained scattered atheromas as isolated lesions. In Grade II, a moderate number of atheromas were present, but they did not exceed 4 to 6 plaques in a 5 × 5 cm. area. Grade III vessels contained numerous atherosclerotic plaques with only a moderate amount of normal endothelium intervening, and Grade IV vessels were those in which the entire endothelial surface was covered with plaques.

The intima of the vessel was carefully dissected from the media, the specimen of intima weighed and suspended in a known volume of saline, and a suspension prepared in a tissue homogenizer. Similar suspensions were prepared from the media and adventitia. As a control, a portion of human brain in an amount equivalent to the vascular tissue was prepared in a similar manner. Following the preparation of the tissue, the suspension was allowed to settle and the particle-free supernate was utilized as a source of thromboplastin in the one-stage prothrombin time. The results of the one-stage prothrombin time obtained with the tissue suspension were compared with the prothrombin time obtained utilizing suspension of human brain as a source of thromboplastin. A thromboplastin generation test was performed in which the suspension of the intima, media, and others were substituted for platelets. The thromboplastin generating properties of the tissue suspension when substituted in this manner were compared with the thromboplastin generation test utilizing normal platelets, with a suspension of human brain substituted for platelets, and with a saline control.

Results. The prothrombin times obtained utilizing a suspension of the vessels as a source of thromboplastin compared with a suspension of human brain are shown in Table 1. No correlation between the degree of atherosclerosis and shortening of the prothrombin time was observed. Although some of the shortest prothrombin times were obtained with suspensions prepared from arteriosclerotic plaques, they did not differ markedly from the results with normal vessels. Although thromboplastic activity was present in the suspensions prepared from the intima, it was not striking and the values varied widely.

TABLE 1.—PROTHROMBIN TIMES UTILIZING TISSUE SUSPENSIONS AS A SOURCE OF THROMBOPLASTIN

	Prothrombin Time	
	Range Time in Seconds	*Average Time in Seconds*
Normal	14–59	41
Grade I	27–59	40
Grade II	43–62	53
Grade III	28–85	50
Grade IV (Plaques)	17–69	40

Thromboplastic activity was clearly demonstrable in the thromboplastin generation test when the tissue suspensions were substituted for platelets (Fig. 1). The tissue suspensions derived from the intima of normal vessels were capable of substituting for platelets in the thromboplastin generation test, resulting in activity similar to that obtained with normal platelets. Thromboplastic activity was also present with suspensions prepared from the media, but was not as potent as the suspensions of intima. When the thromboplastic properties of the normal intima were compared in the thromboplastin generation test with the arteriosclerotic vessels, the activity was greater in the arteriosclerotic vessels. The thromboplastic material obtained from normal and arteriosclerotic intima retained its

activity when diluted and in some instances, the activity was enhanced by dilution. Witte and Bressel[7] noted an increase in thromboplastic activity in aqueous suspensions of normal vessels when diluted and attributed the enhanced activity to the dilution of an antithromboplastin present in the intima. In our study, enhancement of activity by dilution was not consistently demonstrable. Thromboplastic activity was not related to the presence of particulate matter in the tissue suspension since activity was retained following filtration.

The greatest degree of thromboplastic activity was found in suspensions prepared from the intima. Although the suspension prepared from the media had thromboplastic activity, it was less than that observed with the intima and the adventitia was relatively inactive. Similar observations have been reported by Astrup *et al.*[1] and Witte and Bressel[7].

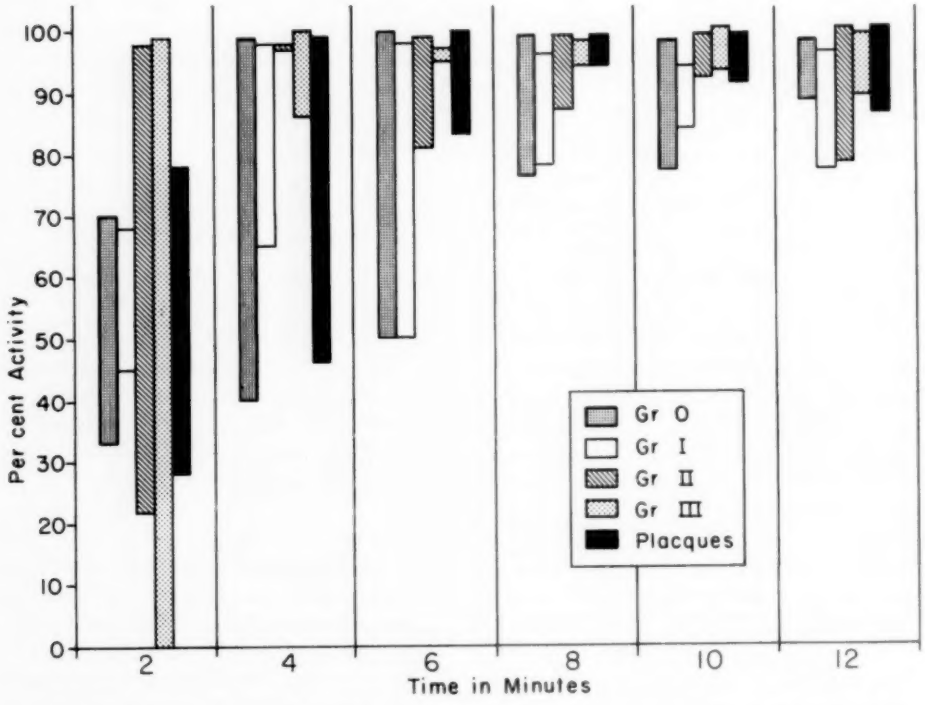

Fig. 1.—The range of clotting times of substrate plasma expressed as % thromboplastin generating activity in the thromboplastin generation test in which suspensions of intima from normal and arteriosclerotic aortas were substituted for platelets.

An attempt was made to correlate the presence of *in vitro* thromboplastic activity of the vessels with the postmortem findings in the individuals from whom the vessels were obtained, but no correlation was present between thromboplastic activity and the presence or absence of intraarterial thrombosis.

A preliminary attempt was made to characterize biochemically the material

responsible for thromboplastic activity in the arteriosclerotic vessels. It was our hypothesis that the ability of suspensions prepared from arteriosclerotic intima to substitute for platelets in the thromboplastin generation test was probably due to the phospholipids present in the atheroma. The ability of the tissue suspensions to substitute for platelets in the thromboplastin generation test is completely destroyed by extracting the tissue suspensions with acetone and, since phospholipids are insoluble in acetone, it seems unlikely that this accounts for the thromboplastic activity. Further studies are in progress to characterize the thromboplastic material.

Discussion. The results of this study indicate that both normal and arteriosclerotic intima possess thromboplastic activity. The coagulant property of the tissues is most readily demonstrated by the ability of saline suspension of the intima to substitute for platelets in the thromboplastin generation test. Astrup *et al.*[1] have demonstrated potent thromboplastic activity in the intima and media of normal and pathologic aortas based on the ability of saline suspensions of these tissues to clot recalcified plasma. Varying degrees of thromboplastic activity have also been found in the normal aorta by Witte and Bressel[7] utilizing a similar technique. In contrast to the findings of Astrup *et al.*[1], we could not demonstrate striking thromboplastic activity in normal or arteriosclerotic aorta based on the ability of the tissue suspension to clot recalcified plasma. It should be pointed out, however, that the method of preparation of the tissue suspension and the substrate plasma utilized in the one-stage prothrombin time differ in our studies from that of Astrup *et al.* The differences in technique may account for the discrepancy in results. It seems clear, however, from this and the previously cited investigations that the intima of the normal aorta has clot-promoting properties.

The thromboplastin generating property of normal vessels is enhanced in arteriosclerotic vessels. The increased activity of the intima derived from arteriosclerotic vessels could result either from a reduction of an antithromboplastin reported to be present in the normal intima by Witte and Bressel[7], or represent an inherent property of the atheroma. Although the data is not available from this study to differentiate between these two mechanisms, the latter would seem to be the most likely explanation.

The demonstration of enhanced coagulant activity in arteriosclerotic vessels may be of significance in elucidating the pathogenesis of intraarterial thrombosis. The functional consequences of arteriosclerosis are generally considered to result from the obstruction of normal vascular lumen with a subsequent decrease in blood flow. This study indicates that atheromas, no matter how trivial, contain clot-promoting activity capable of initiating thrombosis. One of the factors which precipitates thrombosis in arteriosclerotic vessels may be the thromboplastic material present in the arteriosclerotic lesion; hence, thrombosis may be attributable to biochemical as well as mechanical effects of atherosclerosis. The precipitation of thrombosis in arteriosclerotic vessels due to the clot-promoting properties of the atheroma is a factor subject to control by anticoagulant therapy.

Summary. The coagulant activity of the intima and media of normal and arteriosclerotic aortas has been determined by the ability of saline suspensions of these tissues to clot recalcified plasma and to substitute for platelets in the thromboplastin generation test. Thromboplastic activity is demon-

strable in the intima and to a lesser degree in the media, primarily by the ability of the tissue of suspensions to substitute for platelets in the thromboplastin generation test. Thromboplastic activity was enhanced in arteriosclerotic vessels. It is suggested that the clot promoting activity of the atheroma may be a factor in the pathogenesis of intraarterial thrombosis.

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SUMMARIO IN INTERLINGUA

Le Proprietates Coagulatori de Aortas Normal e Arteriosclerotic

Le activitate coagulante del intima e del media de aortas normal e arteriosclerotic esseva determinate in terminos del capacitate de suspensiones salin de ille tissus de coagular recalcificate plasmas e de prender le placia de plachettas in le test del generation thromboplastinic. Activitate thromboplastic es demonstrabile in le tunica intime e, minus pronunciatemente, in le tunica medie, primarimente per le capacitate del tissu in suspensiones de prender le placia de plachettas in le test de generation thromboplastinic. Le activitate thromboplastic esseva plus forte in vasos arteriosclerotic. Es proponite le these que le activitate coagulo-promotori del atheroma es possibilemente un factor in le pathogenese de thrombosis intraarterial.

THE CLINICAL INTOXICATION INDUCED BY A HEPARINOID

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The subject of heparin, synthetic substitutes for heparin and their clinical application remains one of considerable interest.

Some time previously[1] this laboratory reported its observations of the effects of a new heparinoid on a series of control patients. The agent is mepesulfate†, a polyhexuronic acid ester derived from pectic acid. The initial trials with single doses in 200 hospitalized patients were primarily for the purpose of determining the regimen for therapeutic trials. Subsequently the agent was used as an anticoagulant in 83 patients. A relatively long-term follow-up was made necessary because of some of the unusual side effects observed. A summary of these observations is presented at this belated date because of the continued and increased importance of the use of heparin and the relevance of the data to be given.

Material and Methods. EXPERIMENTAL. The techniques utilized to measure the anticoagulant activity of the polyhexuronic acid ester have been previously described in detail (Field *et al.*[1]).

The patients treated in this study were admitted to the wards of the Los Angeles County General Hospital and were unselected as to age and sex. The attending and resident staff initially examined the incoming patients and proceeded with diagnostic and therapeutic procedures in its usual fashion. However, when the diagnosis and circumstances indicated the need or desirability of heparin anticoagulant therapy, the project team assumed immediate responsibility for administration of the drug, the necessary control procedures, and follow-up observations. In most instances, therapy began within several hours after admission to the hospital.

Following discharge of the patient from the hospital, follow-up was accomplished by establishing a special clinic for re-visits or maintaining contact with the patient by correspondence or telephone for almost one year after concluding therapy, or both.

The polyhexuronic acid ester was administered in an isotonic solution with preservatives (Field *et al.*[1]).

THERAPEUTIC REGIMEN. It is arbitrarily accepted that a beneficial and significant inhibition of coagulation is obtained when the whole blood clotting time is prolonged more than 50 and up to 75% beyond that of the control clotting time.

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†Mepesulfate is the generic designation of the sodium salt of sulfated polygalacturonic acid methyl ester methyl glycoside developed in the laboratories of Hoffmann-La Roche, Inc., Nutley, N.J. and furnished by Dr. Leo A. Pirk.

It had already been demonstrated that approximately 250 mg. of the polyhexuronic acid ester given intramuscularly produced a therapeutic response which persisted from 12 to 24 hours (Field *et al.*[1], Zinn, Field and Griffith[7]). Thus the clotting time was always taken prior to any treatment for control purposes and the selected dose of polyhexuronic acid ester given immediately thereafter. The clotting times were done once or twice daily but usually at the same time 24 hours apart. The drug was given in doses of 100 to 500 mg. twice daily, or from 500 to 750 mg. once daily. The goal of maintaining the clotting time at about 50 to 75% above the control was easily achieved in most patients. When clotting times became progressively prolonged or the blood was relatively incoagulable, the drug was temporarily discontinued and resumed at the same or a lower dose.

Results. NATURE OF DISEASES TREATED. The majority of patients, requiring anticoagulant therapy, who entered the wards of this general hospital were, as would be expected, primarily those with existing or impending myocardial thromboembolic disease. In Table 1

TABLE 1.—DISEASE STATES TREATED WITH ANTICOAGULANT

Diseases	*Number of Patients*
Proven myocardial infarction	53
Suspected myocardial infarction	11
Thrombophlebitis	4
Embolism	4
Cardiac disorder (angina pectoris, congestive failure, *etc.*)	9
Others	2
Total	83

there is listed the number of patients in each disease category who were recipients of therapeutic quantities of the polyhexuronic acid ester. Immediate anticoagulant therapy was initiated where myocardial infarction was suspected so that 11 patients received treatment until the attending staff was satisfied by the customary clinical and laboratory examinations that this was not the case and treatment was suspended. All of the remaining 72 patients had proven disease as indicated, the greatest number having a myocardial infarction.

TABLE 2.—DISTRIBUTION OF PATIENTS ACCORDING TO DURATION OF THERAPY WITH ANTICOAGULANT

Duration (in days)	*Number of Patients*
1–2	12
3–7	46
8 plus	25
Total	83

DURATION OF THERAPY. The patients were maintained on the anticoagulant agent for varying periods of time.

TABLE 3.—DISTRIBUTION OF PATIENTS ACCORDING TO TOTAL DOSE OF ANTICOAGULANT

Total Dose of Anticoagulant	*Number of Patients*
Less than 1 gm.	4
From 1 to 5 gm.	54
From 5 to 10 gm.	15
More than 10 gm.	10
Total	83

In some instances, therapy was discontinued as soon as it was evident that no disease existed. In others, the polyhexuronic acid ester was used preliminary to coumarin anticoagulants; and usually within 24 to 48 hours the heparinoid was no longer needed. In Table 2 is given the arbitrary grouping of patients by interval of time. Some of the patients required and received the anticoagulant for up to 30 days continuously.

QUANTITY OF DRUG ADMINISTERED. In Table 3 is given a summary of the total amount of drug administered to the patients.

REGIMEN OF THERAPY UTILIZED. In Table 4 is given a summary of the various combinations of therapy evaluated and the arbitrary evaluation of the anticoagulant effectiveness. The standards for this table were based on the following:

Excellent. The whole blood clotting time was prolonged by at least twice the control value within 12 to 24 hours and was maintained within the range of two to four times this value in more than 75% of the subsequent clotting time tests.

TABLE 4.—EVALUATION OF REGIMEN OF THERAPY OF ANTICOAGULANT AND CLINICAL RESULTS

Dose	*Frequency*	*Total No. Trials*	*Response**		
			Excellent	*Fair*	*Poor*
100 mg.	twice daily	1		1	
250 mg.	once daily	1		1	
250 mg.	twice daily	23	14	8	1
300 mg.	once daily	1		1	
300 mg.	twice daily	15	6	9	0
500 mg.	once daily	19	4	15	0
500 mg.	twice daily	18	1	7	10
750 mg.	once daily	5	0	2	3
	Totals	83	25	44	14

*These criteria are defined in the text.

Fair. The whole blood clotting time was prolonged by at least 50% of the subsequent clotting time tests.

Poor. It was impossible to maintain the whole blood clotting time within the range of two to four times the control value and within a trial period of 4 to 7 days the regimen had to be abandoned.

EFFECTIVENESS OF DRUG AND CLINICAL IMPRESSIONS. The polyhexuronic acid ester was simple to use and in general, its anticoagulant effects were predictable. In almost all instances the anticoagulant activity was demonstrably within therapeutic range within 12 hours and was easily maintained by the intramuscular route by giving usually 250 mg. twice a day or 500 mg. once daily. There were no subjective complaints from the patients. In Table 4, again by arbitrary standards, is given an evaluation of the drug as an anticoagulant by the techniques described. Although no effort was made to obtain a direct comparison, it was felt that the results obtained with the heparinoid were comparable with heparin.

SIDE EFFECTS OBSERVED. The discovery and evaluation of the observations to follow evolved from the diligence of continued patient supervision. An indication of the disposition of patients after therapy is given in Table 5. Of

TABLE 5.—EVALUATION OF EFFECTIVENESS OF FOLLOW-UP

	Number of Patients
Deaths	27
Necropsies	8
Adequate follow-up	40
No possible follow-up	16

the total of 27 deaths, 24 were unequivocally due to the disease being treated. The several unpredicted side effects

became evident on both short and long term observation. Two of these were serious and had critical consequences, one producing death in at least 2 patients. With the recognition of these phenomena, the addition of new patients to this series was immediately discontinued and other investigators notified so that further clinical trials elsewhere were terminated.

TABLE 6.—TOTAL INCIDENCE OF ALOPECIA IN ANTICOAGULANT-TREATED PATIENTS

Alopecia	*Number of Patients*
None	7
Moderate	4
Marked	26
Died during therapy period	27
Lost to adequate (6 week) follow-up	19

of this association is presented. Ultimately more than 70% of patients who survived, and could be accounted for at least 4 weeks after completing therapy, had varying degrees of loss of hair. The average interval until alopecia was clearly manifested was 7 weeks. The majority of these had loss of more than 75% of the central crown of hair up to complete baldness (Figs. 1 and 2). There appeared to be no loss of axillary, pubic, body or eyebrow and eyelid hair and only slight loss of the fringe and side hair of the scalp. In general this state persisted for one or two months (the longest was 5 months) when new hair reappeared and ultimately the new growth was superior in thickness and appearance to the original scalp hair. The recovery from the alopecia was 100%.

TABLE 7.—THYROID STUDIES DONE IN PATIENTS WITH ALOPECIA FROM DRUG THERAPY*

Total Dose of Anticoagulant	*PBI*	*I_{131} Uptake*	*Cholesterol*
Normal	3–8 mg.	10–40%	160–260 mg./100 ml.
11.95 gm.	5.6	26.1	—
11.75	7.0	—	213
9.75	6.1	23.2	224
9.00	7.0	—	—
9.00	6.4	24.0	—
3.50	5.2	—	—
3.00	5.0	17.0	213
2.00	5.0	23.0	—
1.75	4.8	15.3	—

*Each line of results has been obtained from a single patient.

ALOPECIA. The loss of hair by patients would not have been observed had there not been arrangements made for clinic re-visits or by contact for long term follow-up of the treated patient. In most instances some slight loss of hair began about 3 to 4 weeks after treatment had been completed and practically no patient directly attributed this to his drug therapy. However, in time the conclusion became inescapable. In Table 6 the incidence

It was of further interest to note that although there was relationship between the dose and duration of administration of the polyhexuronic acid ester and the ensuing alopecia, loss of hair occurred in several patients after only 2 to 3 days of treatment and a total dose of only 1.5 gm. In general, however, the more extreme alopecia occurred in patients who received 3 gm. or more. That the drug had not produced this effect by inducing hypo-

Fig. 1.—Photograph of scalp of patient with extensive alopecia 4 weeks after treatment with mepesulfate.

Fig. 2.—Microphotograph of biopsy from scalp of patient with alopecia seen in previous figure.

thyroidism was proven by thyroid function evaluation as presented in Table 7.

DIARRHEA. A more severe and also somewhat obscure toxic reaction with fatal consequences was diarrhea. This usually began after 4 to 6 days of therapy (the average interval from therapy until onset of symptoms was 3 weeks) as frequent soft to loose stools; and usually in patients who had been given about 6 to 10 gm. of the anticoagulant. One patient, however, given a total dose of only 1.75 gm. had the onset of frequent watery bowel movements. In Table 8 is summarized the occurence of diarrhea in 15 of 58 patients who survived a satisfactory observation period; an incidence of approximately 25%. Even with discontinuance of the drug and return of clotting times to normal, the diarrhea persisted in several patients and produced severe consequences in others. In some, amebic dysentery and other enteric diseases were suspected because of the indolent nature of the initial attack. After several weeks of these symptoms, 3 patients expired. Postmortem examination of the entire intestinal tract revealed superficial ulcerations, hyperemia of the mucosa, and, in some areas, a pseudomembrane or some hemorrhage into the mucosa. The pathological microscopic description of the small and large bowel of one patient is described as follows, "The epithelial surface is covered in areas by a wide zone of exudate containing fibrin and many neutrophils along with less numerous round cells. The surface epithelium is not seen generally. Many glands are enlarged, round, and show obstructed outlets and contain many inflammatory cells, chiefly polymorphonuclear, and some show large foamy cells with nuclei compressed against one surface. The submucosa shows a round cell infiltration with many lymphocytes, plasma cells, and polymorphonuclears including many eosinophils. Granulation tissue appears to be present in some areas of mucosa with fibroblasts." (For this report we are indebted to Grace Walla, M.D., Department of Pathology, Los Angeles County Hospital).

TABLE 8.—INCIDENCE OF OTHER SECONDARY EFFECTS WITH ANTICOAGULANT THERAPY

Complaint	*Degree*	*Number of Patients*
Diarrhea	Severe	10
	Moderate	3
	Mild	2
	None	43
Skin eruption	Severe	1
	Moderate	2
	Mild	1
	None	29
Bleeding	Mild to moderate	15
	None	33

OTHER SIDE EFFECTS. There were observed some mild, and, never severe, instances of purpuric nature in about 30% of patients on medium to long term therapy. This, in the main, consisted of microscopic, occasionally gross, hematuria and in all cases was associated with prolonged clotting times. Reducing or temporarily discontinuing the anticoagulant led to a disappearance of the hematuria.

A small number, about 12%, of treated patients exhibited evidence of a mild maculopapular generalized body eruption which abated on the discontinuance of the drug.

Two patients also exhibited either mild emesis or some loosening of teeth. The significance of the latter is not clear at this moment.

Discussion. Development of satisfactory heparin substitutes has been complicated by the appearance of toxic phenomena in the case of each new

compound. Paritol (Sorenson and Wright[5]) and Thrombocid (Fisher[3]) are two agents which had to be discarded because of a variety of undesirable side effects.

The initial control and clinical trials with the new heparinoid described herein appeared encouraging (Field *et al.*[1], Field, Zinn and Colovos[2]). Its anticoagulant activity was reproducible and uniform. Management of the patients for moderately long periods of time with the heparinoid was quite satisfactory.

However, the careful observation of patients treated with the drug has revealed several serious noxious effects which make it unacceptable for clinical use.

More than 70% of the patients developed an alopecia; about 25% a diarrhea and about 12% a skin eruption.

The hair loss observed was similar to that found in adult male baldness of the hereditary type. It was most pronounced in the fronto-parietal and crown areas. Other drugs also produce alopecia. Thallium, (beta-diethylaminoethoxy) phenyl-phenethyl ketone, 5-fluorouracil and several of the anticancer agents induce a diffuse hair loss without definite pattern. In general, knowledge of baldness is very limited. Hereditary factors in the presence of the male hormone are the only definite prerequisites.

Microscopic examination of biopsy specimens taken from the scalps of treated alopecic patients revealed findings typical of those observed in natural male baldness (Fig. 1). A typical specimen (Fig. 2) exhibited an increase of the sebaceous apparatus. This may be due to replacement of the follicles by the sebaceous glands.

Although alopecia has several times previously been ascribed to the synthetic compounds (Fisher[3], Fisher, Bircher and Reich[4]), heparin itself in large doses, and even the coumarin class of drugs, have been incrimated in producing loss of hair (Fisher, Bircher and Reich[4]). In the face of the widespread use today of both heparin and the coumarin derivatives, it would be expected that the phenomenon would have been reported with some frequency. To the contrary, loss of hair has not been observed by several of the clinical groups supervising large scale trials (Dr. Irving S. Wright, personal communication).

Apparently prophylactic administration of vitamin D_2 served to prevent loss of hair in patients treated with Thrombocid (Thomascheck[6]).

The present report is the first to indicate not only the high incidence of delayed loss of hair stemming from even a relatively small dose of a polyhexuronic acid ester heparinoid but also the phenomenon of an ulcerative change of the intestinal tract with irreversible diarrhea. This more serious consequence delimits the clinical trial at least of this one agent, but should alert investigating groups developing newer heparinoids to this threat.

The search for heparin substitutes will continue because of the clinical value of heparin as both an anticoagulant and antilipemic agent. However, the catastrophic experiences with previous polyhexuronic derivatives leaves doubt that there would be value in pursuing further synthesis of analagous agents. The continued difficulty of providing an inexpensive source of heparin, however, will justify explorations designed to produce some other type of substitute for it.

Summary. A synthetic heparinoid, mepesulfate, a polyhexuronic acid ester derived from pectic acid, has been administered to 83 patients as specific anticoagulant therapy.

Large intramuscular doses of mepesulfate, usually given twice daily, pro-

duced a satisfactory anticoagulant effect with excellent ease of control of management for varying periods of time. It appeared that the results obtained were comparable with heparin itself.

However, several serious systemic effects were observed which made the agent unacceptable for clinical use. About 70% of surviving patients experienced an alopecia as an effect delayed several weeks after onset of therapy. Recovery from this was 100%.

Approximately 25% of treated patients manifested a diarrhea of from mild to severe degree. In several instances the diarrhea continued after discontinuance of therapy and was associated with 3 deaths. Necropsy examination showed a generalized ulcerative enterocolitis.

A small number of patients manifested evidence of some purpura and some had a mild maculopapular eruption.

ACKNOWLEDGMENT: We note with appreciation the technical assistance provided by Ray Hartwig, M.D.

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SUMMARIO IN INTERLINGUA

Le Intoxication Clinic Inducite per Un Heparinoide

Un heparinoide synthetic, mepesulfato, un estere de acido polyhexuronic derivate ab acido pectic, esseva administrate a 83 patientes como specific agente de therapia anticoagulante.

Grande doses intramuscular de mepesulfato, usualmente administrate duo vices per die, produceva un satisfacente effecto anticoagulante con excellente simplicitate de regulation durante varie periodos de tempore. Il pareva que le resultatos obtenite esseva simile a illos de heparina mesme.

Tamen, plure series effectos systemic esseva observate le quales rendeva le agente inacceptabile pro uso clinic. Circa 70% del supervivente patientes experientiava un alopecia como effecto tardive del therapia, comenciante plure septimanas post su initiation. Le restablimento ab isto esseva 100%.

Approximativemente 25% del patientes tractate manifestava un diarrhea de grados leve a sever. In plure casos le diarrhea continuava post le cessation del therapia e esseva associate con 3 mortes. Le necropsias monstrava un generalisate enterocolitis ulcerative.

Un basse numero de patientes manifestava signos de un certe grado de purpura, e plures habeva un leve eruption maculopapular.

LIPEMIA CLEARING EFFECT OF BUCCALLY ADMINISTERED HEPARIN POTASSIUM (CLARIN)*

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The lipemia-clearing action of parenterally administered heparin has been demonstrated and confirmed by many investigators (Hahn[7], Korn[8], Shore, Nichols and Freeman[13]). Others (Engelberg[3], Engelberg, Kuhn and Steinman[4], Frederickson[5], Kuo and Joyner[10]) have suggested the use of this lipoprotein lipase activator as a factor in the interruption of atherogenesis and the treatment of the sequelae of this disorder; that is, coronary artery disease and myocardial infarction. Since buccal or sublingual administration is normally preferable to parenteral, it seemed desirable to add clarification to the differing reports (Berkowitz, Likoff and Spitzer[1], Engelberg[2], Fuller[6], Litarius *et al.*[11], McDevitt, Huebner and Wright[12]) as to the efficacy of heparin as a buccally-absorbed lipemia clearing agent. The present study is designed to assay the prevention of fat-induced postprandial hyperlipemia by both buccally and parenterally administered heparin. We have also attempted to evaluate the response to buccal tablets of different pharmaceutical composition both as to rapidity of effect and duration of action.

Procedure and Methods. A total of 34 patients was studied to determine the effect of a fat challenge upon their serum total lipid levels. This procedure was then repeated 15 minutes following 6,000 units of intravenously administered heparin and a third time after the administration of 6,000 units of heparin potassium (Clarin) buccally. Twenty-four of the group were given a Clarin preparation (series 1) that took longer than 15 minutes to disintegrate and 11 (series 2) received a tablet that disintegrated in less than 10 minutes in the buccal pouch. The "fat meal" given to each subject after a 12-hour fast consisted of two soft boiled eggs, one slice of dry toast, black coffee, and 6 ounces of heavy sweet cream. Pre-treatment (fasting) control samples were drawn and the serum obtained. Further specimens were taken at 150- and 300-minute intervals. The total lipids were determined by the method of Kunkel, Ahrens and Eisenmenger[9].

Upon inspection of the data presented for analysis, it appeared probable that we were dealing with two types of response to the experimental conditions. The design of the experiments utilize the response of patients' total blood lipid values to a fat meal challenge. These experiments attempt to discover what effects on that response are exerted by substances said to promote the appearance in the systemic circulation of "lipoprotein lipase" or "clearing factor." An additional variable is introduced by the fact that the two substances are administered by different routes. Clearing factor activity is thought to be equal with the two preparations.

Apart from the normal variability of lipid responses and the procedural (analytic) error to be expected, these experiments confirmed the suspicion that there is an idiopathic predisposition among patients of whatever diag-

*Thos. Leeming & Co., Inc.

nosis toward differing responses to conditions such as were here imposed. Looking at the control determinations only (no treatment), it was obvious that some patients were responsive to the fat meal challenge in that the total lipid levels rose and remained elevated at 2½ hours and 5 hours after the fat meal challenge. Other patients either showed no response at all to the challenge, or were much less responsive both as to quantitative rise or duration of response. It was decided to attempt to establish some logical, mathematically germane test which would assign these patients to their appropriate groups.

Since the absolute levels involved were individually specific and varied widely, we employed the per cent increase of each experimental value (after fat meal) as reflected against the fasting value. Using the entire group, we obtained the mean of all percentile responses as described and obtained the standard error of that mean. It has long been known that a rough range for significance of mean values may be obtained by adding twice the standard error to that mean (Hahn[7]). This range was established for the groups in series 1 and 2. The test required that both determinations after the fat meal challenge should minimally fall within the range obtained. Values which exceeded the high end of the range were retained, while the presence of one value below the low end of the range was deemed sufficient to assign the particular patient to what we have called the "lipid stabile" group. Those patients who met the criteria have been called lipid labile, and the results of their studies are presented in this report.

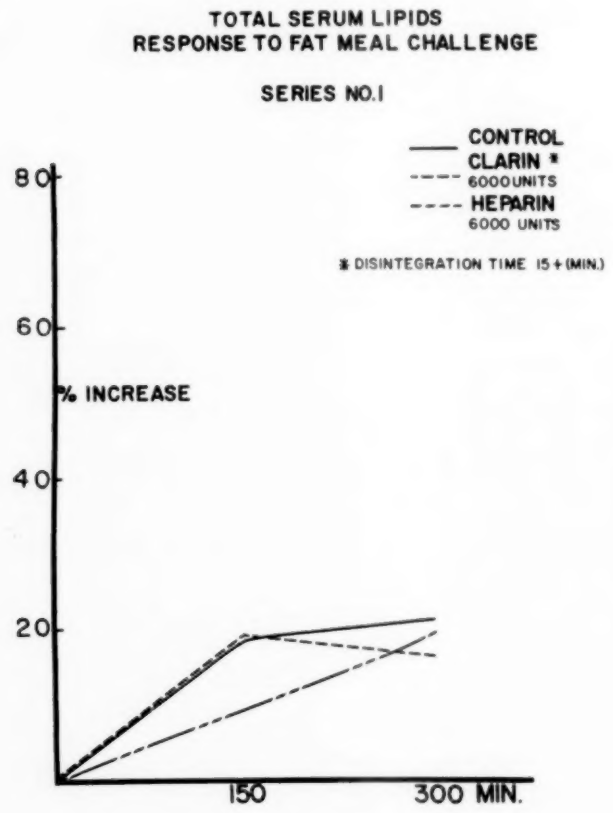

Fig. 1

TABLE 1.—EFFECT OF HEPARIN ADMINISTRATION ON TOTAL SERUM LIPIDS (AFTER FAT MEAL CHALLENGE)

Series No. 1 *Treatment*	*Time*	*Rt.*	*% Inc.(Av.)*	*± St. Error*	*'P'*	*Cases*
Control	150 min.	—	18.00	3.16	—	9
Control	300 min.	—	21.00	3.39	—	9
Clarin	150 min.	Buccal	19.00	4.63	0.1	9
Clarin	300 min.	Buccal	15.90	3.68	0.05	9
Heparin	150 min.	I.V.	9.60	2.54	0.01	9
Heparin	300 min.	I.V.	19.60	3.93	0.1	9
Series No. 2						
Control	150 min.	—	39.60	20.70	—	4
Control	300 min.	—	19.70	1.60	—	4
Clarin	150 min.	Buccal	17.70	5.00	0.1	4
Clarin	300 min.	Buccal	19.80	4.30	0.1	4
Heparin	150 min.	I.V.	11.10	7.90	0.1	4
Heparin	300 min.	I.V.	11.50	4.30	0.01	4

Note: Series No. 1—Clarin Disintegration >15 min.
Series No. 2— " " <10 min.

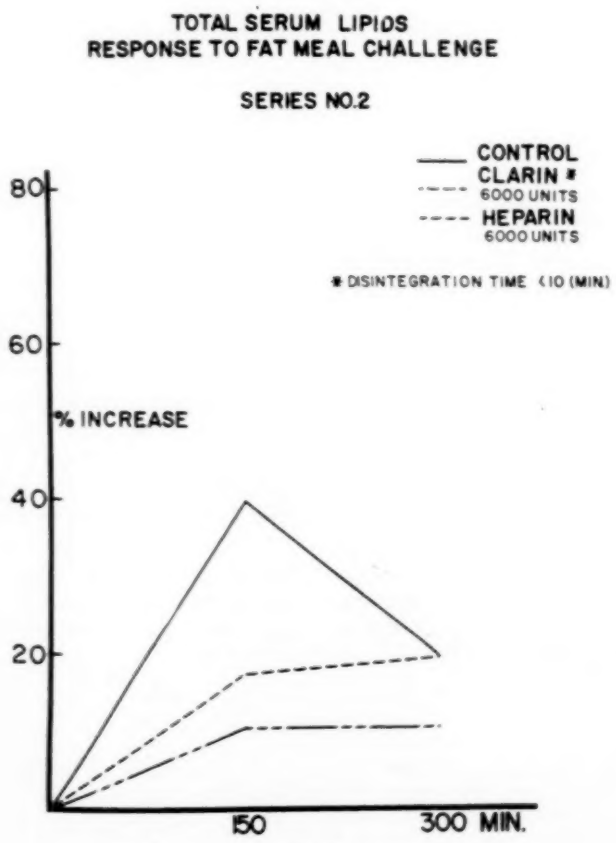

Fig. 2

Results. Series No. 1 reveals that buccal Clarin is significantly effective in inhibiting the rise in lipid concentration only at the 300 minute time interval while intravenous heparin was more effective only at the 150 minute sampling.

Series No. 2 indicates some change in the onset of action of buccal Clarin when a change in physical composition of the tablet hastens disintegration time. Although the number of patients in the experiment is small, Clarin treatment approached significance at the 150-minute interval. No significant effect was noted at the 300-minute interval in this series. Intravenously administered heparin was found effective in inhibiting the expected total lipid rise at both time intervals.

Discussion. Investigations of Clarin in the past have utilized optical density determinations (Fuller[6]) and biochemical estimations of the lipoprotein lipase activity (Engelberg[2]). We believe that the determination of total lipids to have advantages over either of these other means of investigations. The former does not take into account that in the postabsorptive state, triglyceride moieties travel in the blood as part of low-density lipoprotein molecules that are not large or numerous enough to affect the gross clarity of the serum (Van Itallie and Felch[14]). The latter method is fraught with peril when used in this type of investigation, owing to the precise controls of temperature, pH, and time which must be exercised. Since facilities for this type of analysis are not generally available, the use of the simple more direct technique of measuring a single known material (Kunkel, Ahrens and Eisenmenger[9]) in the serum seems additionally justified.

The increased incidence of coronary atherosclerosis in patients with essential hyperlipemia, the presence of elevated serum triglyceride levels, and an impairment of the rate of alimentary lipemia clearing (Van Itallie and Felch[14]) are all indications for the use of heparin in these disorders. The experiments presented in this paper indicate the feasibility of utilizing mucosally-absorbable lipemia clearing preparations in subjects with atherogenetic stigmata and present a simple method for evaluating their therapeutic efficacy.

Summary. 1. Administration of a fat meal produces an acute elevation of serum total lipid levels.

2. Utilizing statistical procedures, it is possible to separate the lipid response pattern of individuals into those who make a valid response to the fat challenge test and those who do not.

3. It is practical to use a fat challenge technique in the evaluation of materials acting upon the metabolism and transport of lipids. These appraisals can be made relative to the type, onset and duration of action of the materials.

4. Buccally and parenterally administered formulations of heparin potassium exert an inhibiting action on the elevation of serum total lipids ascribable to a fat challenge meal.

5. The time interval at which the buccal preparation acts demonstrates that its effectiveness is in part dependent upon the disintegration time of the tablet.

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SUMMARIO IN INTERLINGUA

Le Effecto Acclaratori Exercite Super Lipemia per le Administration Buccal de Heparina a Kalium (Clarin)

1. Le administration de un repasto grasse produce un elevation acute in le nivellos seral del lipidos total.

2. Es possibile, per le utilisation de technicas statistic, de distinguer duo modos de reaction lipidic secundo que le subjectos manifesta un reaction valide al test de provocation a grassia o non.

3. Es practic usar un technica de tests de provocation per grassia in le evalutation de materiales que age in le metabolismo e le transporto de lipidos. Iste evalutationes pote esser facite relative al typo, al declaration, e al duration del activitate del materiales.

4. Preparatos de heparina a kalium administrate per via buccal e parenteral exerce un influentia inhibitori super le elevation del nivellos seral del lipidos total attribuite a un repasto provocatori de grassia.

5. Le intervallo de tempore post le qual le preparato buccal age demonstra que su efficacia depende in parte del tempore de disintegration del tabletta.

PROGRESS
OF
MEDICAL SCIENCE

THERAPEUTICS

UNDER THE CHARGE OF

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SYMPATHETIC NERVE BLOCKING DRUGS FOR THE TREATMENT OF HYPERTENSION*

TM-10, BETA TM-10, BRETYLIUM AND GUANETHIDINE

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THE last two years mark a new era in the treatment of essential hypertension. Compound TM-10, beta TM-10, bretylium and guanethidine were discovered and subsequently used to block the sympathetic nervous system in patients with essential hypertension. The purposes of this article are to review the events leading to this new era, and to discuss the pertinent pharmacology of these four drugs. This is possible because close to a hundred articles have been published, a rate unprecedented in the growing literature on the therapy of hypertension. It should be stated at the outset that no attempt will be made to state as to which drug is better than the other. Such decision depends on a properly controlled study in a group of patients receiving one of five agents: a placebo, TM-10, beta TM-10, bretylium and guanethidine. Unfortunately such a study has not yet been completed.

1. CLASSES OF SYMPATHETIC BLOCKING DRUGS. The classical definition for a *sympathetic or adrenergic blocking agent* is one that "selectively inhibit(s) the responses of effector cells to adrenergic sympathetic nerve impulses and to epinephrine and related amines"[41]. The use of two other terms, *sympatholytic* (blockade of responses to sympathetic nerve impulses) and *adrenolytic* (blockade of responses to epinephrine and related amines) has

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been criticized[73] because the distinction between both types of actions has been largely artificial. Almost all known sympathetic blocking agents are more effective in antagonizing circulating epinephrine than in blocking responses to sympathetic nerve activity, and the latter action could be elicited if large enough doses were used. All sympathetic blocking agents can manifest both sympatholytic and adrenolytic actions, so that classification of drugs into one or the other category has not been possible. This generalization refers to the following drugs: β-haloalkylamines including Dibenamine and phenoxybenzamine (Dibenzyline), ergot alkaloids including dihydrogenated derivatives, imidazolines including tolazoline (Priscoline) and phentolamine (Regitine), dioxane derivatives including piperoxan (933 F; Benodaine), and dibenzazepine derivatives including azapetine (Ilidar)[41,42,73].

In 1954, Hey and Willey[47] discovered a unique feature of TM-10; that is, this compound blocks sympathetic nerve stimulation more conspicuously than injected catecholamines. The chemical structures of TM-10, as well as of the other compounds that have been introduced, are listed in Fig. 1. There now appears to be a definite qualitative difference between this new class of sympathetic blocking drugs, and the older ones. This new class of drugs has been referred to as sympatholytic, antiadrenergic, neurone blocking, and sympathetic nerve blocking. The last term will be used throughout this article.

2. TM-10: A PROTOTYPE FOR SYMPATHETIC NERVE BLOCKING AGENTS. A. *Extent of Autonomic Blockade:* The intravenous injection of sufficient amounts of TM-10 has been shown to block the effects of electrical stimulation of the following sympathetic nerves: cardioaccelerator nerve[34], vasoconstrictor fibers of the hepatic nerve[1], preganglionic and postganglionic nerves to the nictitating membrane[47], splenic nerves which release norepinephrine[34] and contract the splenic capsule[72], bronchodilator nerve fibers[55], and hypogastric nerves which relax the uterus[47]. All of these results establish the ability of TM-10 to block the effects of sympathetic nerve stimulation. The

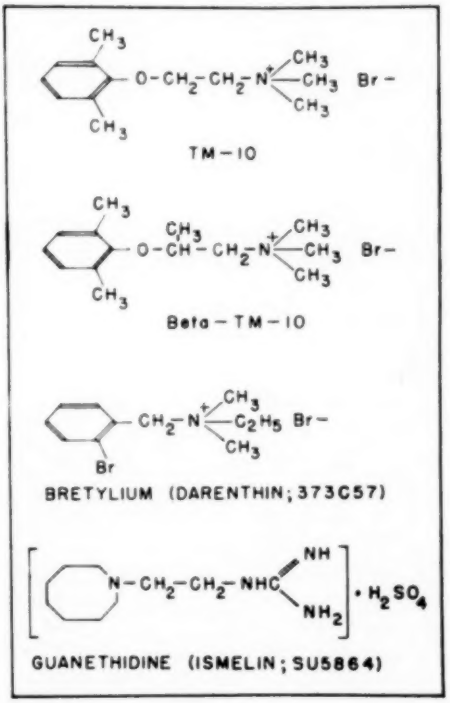

Fig. 1

preferential action on sympathetic nerves is based on the following additional observations: The pressor action of injected epinephrine or norepinephrine in the spinal cat may be transiently reduced but the sympathetic nerve blockade may persist up to several hours[47,89]. This is the major distinction between TM-10 and the classical sympathetic blocking drugs. There is some evidence for ganglion blocking action,

both sympathetic and parasympathetic[31,34,46] as well as curareform action[89] but these additional actions are usually encountered when TM-10 is injected in doses larger than that necessary for sympathetic nerve blockade.

B. *Mechanism of Action.* The exact details of blockade of sympathetic nerve induced by TM-10 is not yet completely known. The earliest explanation was that offered by Hey and Willey[47] that TM-10 caused a local anesthetic action and therefore suppressed conduction in postganglionic sympathetic nerve fibers. Exley[34] failed to show any significant impairment of conduction of impulses along such nerves and substituted a second explanation. Compound TM-10 reduced the amount of transmitter liberated on stimulation of adrenergic nerves by interfering with the biosynthesis of catechol amines. The depletion of catecholamines was demonstrated in rat adrenal medulla[23,31]. The manner of interference of synthesis is not yet clear. The formation of dopamine is prevented[7] as well as the transformation of dopamine to norepinephrine[39], but other possible pathways have not yet been excluded. Inhibition of amine oxidase[18] has been demonstrated but this does not appear to be related to the synthesis of catecholamines.

C. *Undesirable Features.* This prototype of a sympathetic nerve blocking agent has been used largely in animals. Its trial in man[6,39] revealed undesirable actions, predominantly muscarinic in nature. This includes salivation[18], contraction of intestinal wall[31,89], and vasodilatation[31]. There is a latent period of up to one hour for the onset of nerve blockade which renders the use of the drug difficult in most acute experiments.

3. BETA-METHYL ANALOG OF TM-10. McLean and his associates[65] have tested several analogs of TM-10. The beta-methyl analog (see Fig. 1) is unlike TM-10 in that the former is devoid of muscarinic or parasympathomimetic actions. However, like TM-10, the beta-methyl analog retains all the sympathetic nerve blocking actions such as: relaxation of nictitating membrane in unanesthetized cat[65]; no reduction in cervical postganglionic action potentials[65]; reduction in heart rate and blood pressure of anesthetized cats and dogs[66]; inhibition of pressor response to common carotid occlusion[66].

There are some other observations which confuse the otherwise simple actions of beta TM-10. Beck and his collaborators[10,80] have demonstrated blockade of the vasoconstrictor effect of electrical stimulation of the lumbar sympathetic chain, but not the vasodilator effect. The vasodilator fibers, at least to the extremities of the dog, are not blocked by beta TM-10. The lack of blockade of the effects of injected sympathomimetic amines in intact animals has been challenged in other preparations. In the isolated cat atrium, a monoamine oxidase inhibitor (phenelzine, Nardil) causes stimulation of force of contraction but this is blocked by beta TM-10[64]. Rosenfeld, Straughn and Moyer[79] demonstrated that the beta-methyl analog of TM-10 blocks the pressor effect of an infusion of levarterenol in patients. There appears to be a species difference regarding the "adrenolytic" action of beta TM-10.

4. BRETYLIUM. Several benzyl quarternary ammonium compounds have been tested by Boura, Copp and Green[13] for sympathetic nerve blocking action. One of them, bretylium tosylate or bromide (see Fig. 1) has been tried extensively for the treatment of essential hypertension.

A. *Extent of Autonomic Blockade.* The evidence for sympathetic nerve blockade by bretylium is parallel to

that for TM-10. Bretylium blocks the responses to electrical stimulation of the following sympathetic nerves: cardiac nerve causing acceleration and stimulation of myocardial force of contraction[5,14]; cervical sympathetic inducing vasoconstriction of the rabbit ear[14]; preganglionic and postganglionic cervical sympathetic causing contraction of the nictitating membrane[14]; splanchnic nerve causing relaxation of the duodenum[14]. The blockade by bretylium is more selective than that of TM-10. Unlike the latter, bretylium does not possess even a transient blockade of the pressor response to levarterenol, and instead there is even potentiation of the pressor effect of some sympathomimetic amines[14,44,90]. The blockade of autonomic ganglia and neuromuscular junction by bretylium is less conspicuous than that by TM-10[5,14].

B. *Mechanism of Blockade.* Boura and his collaborators[12] studied the distribution of C^{14}-labelled bretylium in cat tissue. The rise and fall of concentration in sympathetic ganglia and postganglionic sympathetic nerves showed a close similarity to the duration of sympathetic blockade, as manifested by relaxation of the nictitating membranes. They concluded that bretylium may act in a manner analogous to that of the local anesthetic drugs, and its specificity may be related to their selective accumulation in sympathetic neurones. Such a local anesthetic action is similar to that of TM-10 but the effects on biosynthesis of catecholamine for bretylium is not yet known.

C. *Cardiac Effects.* Since bretylium is known to block sympathetic nerves to the heart, it is surprising to note that there is a transient period of cardiac stimulation following the administration of bretylium. This has been demonstrated in the intact dog in which the force of myocardial contraction is increased[5]. Such an effect has been referred to as an isoproterenol-like response of the heart muscle for several reasons: cardiac acceleration can still occur even in an adrenalectomized dog[50]; an increase in contractile force can be elicited in the isolated atrium of the guinea pig[37] and in the dog heart-lung preparation[38]. Gaffney[38] administered reserpine to a group of dogs prior to setting the heart-lung preparation and noted that the positive inotropic action of bretylium was reduced but the positive chronotropic action was reversed to a negative one. These results have been interpreted by Gaffney to indicate that bretylium exerts at least part of its initial cardiac stimulation to a release of catecholamines in the heart. A similar conclusion was reached by Furchgott and Kirpekar[37] in the isolated guinea pig atrium. If bretylium is administered intraperitoneally in rats, the end-result is a reduction in sympathetic nerve activity as evidenced by the reduction in heart phosphorylase activity[45]. The transient stimulation is seen only in high doses and is probably not encountered when the drug is given so that the blood levels do not rise abruptly.

Bretylium caused an increase in cardiac output in the anesthetized dog[5] as well as in the sheep[43]. In man, the intravenous injection of 50 to 150 mg. (total) caused a reduction in cardiac output, measured 40 to 60 minutes after the injection[21,28,71]. This discrepancy is not real but indicates that the immediate increase in cardiac output seen in animals is probably a temporary response related to a sympathomimetic-like action. The persistent reduction in cardiac output is related to blockade of sympathetic nerves to the heart or to the peripheral blood vessels or both.

D. *Vascular Effects.* Boura and his

collaborators described the first successful reduction in blood pressure, in man, induced by bretylium[15]. In normotensive volunteers, the intravenous injection of bretylium caused no effect on blood pressure in the supine position but a reduction in the upright position. In hypertensive patients, this drug reduced the supine blood pressure and caused postural hypotension. The hemodynamic mechanism for the reduction in blood pressure in man has been investigated by other investigators. Doyle, Fraser and Smith[28] measured cardiac output by the indicator dilution technique and noted that the period of hypotension was marked by a reduction in output in 3, and increase in 2 and no effect in 2 subjects. The calculated systemic vascular resistance fell in 3, rose in 1, and was unchanged in 3. These measurements require additional study in a larger series of patients to verify if a fall in systemic vascular resistance, as expected from nerve blockade, is a more frequent response. The experiments so far reported by Doyle *et al.*[28] contain one consistent feature. Prior to the administration of bretylium, tilting from the horizontal to vertical position caused a rise in total vascular resistance, but after bretylium, tilting did not alter vascular resistance.

In experimental animals, the intravenous injection of bretylium caused variable effects on blood pressure. A transient rise may occur, and this is related to the transient cardiac stimulation. Nash[71] reported that the pressor response in rats can be blocked by prior administration of reserpine. This initial rise is not a consistent response in anesthetized dogs and may even be replaced by an immediate fall in blood pressure. The explanation here is probably a local vasodilator action which can be demonstrated if the drug is injected into the arterial supply of various organs[4]. The pulmonary vessels are an exception to this generalization because bretylium has no important local action[3].

Doyle, Fraser and Smith[28] noted in five hypertensive patients that bretylium caused a reduction in glomerular filtration rate and effective plasma flow, while the blood pressure was falling. A reduction has been reported also by Soloff[84]. However, although the blood pressure remained low 2½ to 3 hours later, the renal values had risen to values above the initial levels. This feature is regarded by these investigators to be a definite advantage over ganglion blocking drugs.

Conway[21] reported a transient reduction in digital plethysmogram following the intravenous injection of bretylium (12.5 mg.) in man. This was interpreted to mean vasoconstriction of the skin and was accompanied sometimes by a sharp elevation in blood pressure. This vasoconstriction is difficult to explain by pure blockade of the sympathetic vasoconstrictor nerves. Such an expected action can be observed after the acute phase as persistent vasodilatation, reflected as an increase in temperature of the skin of the hand and foot. Blair, Glover and Roddie[11] infused bretylium directly into the brachial artery of human volunteers and demonstrated blockade of the vasoconstrictor response to the forearm induced by muscular exercise. Litwin, Dil and Aviado[54] demonstrated the blockade of vasoconstrictor response of the dog hindlimb during anoxia and asphyxia.

The blockade of the sympathetic nerves to blood vessels appears to be confined exclusively to the vasoconstrictor nerve fibers. The vasodilator fibers are resistant to bretylium on the basis that the following nervous responses are still retained: vasodilatation by veratridine[5], vasodilatation dur-

ing re-oxygenation following anoxia[54], and vasodilatation by muscular exercise[11]. The adrenal medulla is not blocked by bretylium so that the release of catecholamines can still account for some of the vasoconstrictor responses to cold pressor test in an individual after receiving bretylium[35]. In an anesthetized dog after bretylium, the adrenal medullary mechanism can still account for some cardiac stimulation and hypertension induced by common carotid occlusion, increased intracranial pressure and anoxia[5]. The regulation of the cardiovascular system after bretylium is converted to a predominantly hormonal (adrenal medullary) control and this is the probable status when bretylium is administered in patients with essential hypertension.

E. *Treatment of Hypertension.* The initial report of Boura *et al.*[15] on the use of bretylium to reduce blood pressure in hypertensive patients has been confirmed by several groups. Table 1 summarizes their experiences including oral dosage, efficacy and side effects. Nasal stuffiness should be expected on the basis that the nasal sympathetic vasomotor fibers have been blocked. Postural hypotension and its consequences (dizziness, nausea, fainting) are other manifestation of blockade of sympathetic vasoconstrictor nerves[16]. Two side effects appear to be unrelated to sympathetic nerve blockade: muscular weakness and pain in the parotid region. The former is probably related to a neuromuscular blocking action seen if large doses are administered in animals[70]. The latter is related to a secretagogue action on the salivary gland.

The most undesirable features of bretylium are its poor oral absorption and the development of tolerance[50,52]. Duncombe and McCoubrey[29] measured the extent of oral absorption in human subjects and the values ranged from 7 to 45%. Dollery, Emslie-Smith and McMichael[25] collected 5.1 to 18.3% (av. 12.9 in 10 subjects) of the administered dose from the urine, and noted that absorption was greater if the subjects were fasting. The major route of excretion is by the kidneys. From 65 to 83% of the administered dose can be collected from the urine but the amount collected is reduced to about half in patients with high blood urea levels. Most of the drug is excreted in the urine unchanged using C-14 tagged bretylium[29]. The poor and unpredictable oral absorption accounts for most of the difficulties in the clinical use of bretylium. The inefficacy after its prolonged use was tested by Hurley, Page and Dustan[49] by intravenous injection of bretylium; four patients that were tolerant to oral absorption were able to react to intravenous administration, but two patients were tolerant to both forms.

5. GUANETHIDINE. A. *Extent of Autonomic Blockade.* The sympathetic nerve blocking action of guanethidine has been demonstrated by Maxwell *et al.*[50,62]. The effects of preganglionic nerve stimulation (cervical, splanchnic, and celiac) are either reduced or blocked, but the effects of injected levarterenol can still be elicited. These observations suggest either a ganglion-blocking action or a sympathetic blocking action, and evidence for both types of action was derived from measurement of action potentials in the superior cervical ganglion of the cat. There was suppression of potentials recorded from the postganglionic nerve immediately after the injection of guanethidine, but ganglion transmission recovered within one-half hour. This was followed by a protracted period of normal ganglionic transmission but blockade of postganglionic sympathetic nerve.

The acute transient phase of sympa-

TABLE 1.—CLINICAL TRIAL OF BRETYLIUM IN HYPERTENSIVE PATIENTS

Investigators	*No. of Patients*	*Daily Oral Dose (gm.)*	*Efficacy*	*Other Side Effects**
Boura, Green, McCoubrey, Lawrence, Moulton & Rosenheim[15]	36	0.4 to 2.5	Orthostatic hypotension	
Baird & Ul-Haq[8]	25	0.6 to 2.4	Satisfactory control in 19	Renal failure; psychotic episodes
Clausen, Hilden & Krogagaard[20]	20	0.4 to ?		Ptosis; muscular weakness
Conway[21]	10	0.16 to 3.2	Av. BP fall: supine 11/13; upright 32/22 mm. Hg	Conjunctival injection
Dollery, Emslie-Smith & McMichael[24-27]	50	0.2 to 3.6	Satisfactory in 60%, but not for several months. (reduced by 2.0 mm. diast. or less than 160/90 upright	Nausea
Doyle, Fraser & Smith[28]	25	0.4 to ?	Controlled hypertension in 11 free of side effects; controlled but moderate side effects in 6	Bilateral Horner's; mental depression, headache, tremor
Evanson & Sears[33]	27	0.6 to 1.8	Satisfactory fall in BP in 22	Diarrhea, muscular weakness
Freis, Sugiura & Liptak[35]	16	0.6 to 3.0		
Genest, Dufault, Pigeon, Davignon, Biron & Trudel[40]	41	0.3 to 1.2	Av. BP fall upright 24/15 mm. Hg	Digestive disturbances

Davignon, Biron & Thaler				
Hayden & Boake[44]	16	0.1 to 3.2		Headache, Diarrhea
Hodge & McPhie[48]	9	0.3 to 3.6	Satisfactory initial fall in BP in all	Fatigue; unstable personality
Hurley, Page & Dustan[49]	13	2.0 to 8.0	Weekly diast. less than 103 mm. in 6	Abdominal pain
Mackie[56]	9	0.3 to 2.8	Satisfactory reduction in BP in 8	Bronchospasm
Morris[69]	7	0.6 to ?		
Smirk & Hodge[82]	33	0.2 to 2.8		Bradycardia; muscular weakness; heaviness of eyelids; looseness of bowels
Soloff, Reidenberg, Winters & Bello[83,84]	37	0.3 to ?		Itchy eyelids; free bowel movement; occipital paresthesia; dyspnea; elevated BUN
Stuckey[86]	20	0.6 to 2.1	Av. BP below 100 mm. in 11	Vomiting; diarrhea; muscle pains; headache
Turner & Lowther[88]	40	0.3 to ?		Chest constriction; diarrhea

*Side effects other than stuffy nose, orthostatic hypotension and pain in parotid region.

TABLE 2.—CLINICAL TRIAL OF GUANETHIDINE IN HYPERTENSIVE PATIENTS

Investigators	*No. of Patients*	*Daily Oral Dose (gm.)*	*Efficacy*	*Side Effects*
Arnold & Kaiser[2]	25	0.01 to 0.100	Reduction in systolic blood pressure not lower than 120 mm. in standing	
Bartorelli, Gargano, Regoli & Zanchetti[9]	12	0.04 to 0.1	Reduction in supine and standing position	
Eagan & Orgain[30]	37	0.01 to 0.4	Fall in recumbent BP 20 mm. in 9 and similar fall in standing BP in 22	Diarrhea, abdominal pain, impotence
Frohlich & Freis[36]	15	0.012 to 0.150	Av. fall 7/4% supine and 18/14% in sitting, and 27/26% in erect	Bradycardia, nasal stuffiness, drooping of eyelids, impotence
Leishman[51]	43	0.01 to ?		
Lichtlen, Schaub & Buhlmann[53]	24		Av. fall 26/30%	Diarrhea
Maronde, Barbour, Haywood & Denney[57]	20	0.025 to 0.1	Av. fall 0/9 supine and 24/23 standing	Nausea and diarrhea
Meesmann[67]	31	0.02 to 0.100	Fall in dias. & sys. BP in all	Diarrhea, impotence
Page & Dustan[74]	18	0.025 to 0.450	Decrease in BP both supine and standing in 16; standing BP in 2	Diarrhea

thetic ganglion blockade is accompanied by parasympathetic blockade and sympathomimetic stimulation[61]. The latter was manifested in dogs in which the drug was given intravenously[74]. Electrical impulses in the carotid sinus nerve and splanchnic nerve were not altered after guanethidine[63]. In the heart-lung preparation from a reserpinized dog, guanethidine had a negative chronotropic effect[38].

B. *Mechanism of blockade.* A depletion or norepinephrine level in the hearts of rabbits, cats and rats following guanethidine has been shown by several groups of investigators[17,19,81,85]. The depletion is similar to that observed after reserpine, although slower in onset. It has been suggested that the reduction in blood pressure by guanethidine is by producing chemical sympathectomy through depletion of norepinephrine from peripheral nerve endings. It is not yet certain if the depletion is due either to release or to blocking of its synthesis. Maxwell and his collaborators[60] have developed a thesis that the drug acts directly on smooth muscle but more direct evidence is necessary.

C. *Hemodynamic Studies.* Like bretylium, guanethidine causes a reduction in cardiac output in man, with no significant change in peripheral vascular resistance[78]. An increase in calculated vascular resistance was reported by Lichtlen, Schaub and Bühlmann[53]. Renal blood flow and glomular filtration rate were also reduced but there was no oliguria[22,77,78]. Total renal vascular resistance was decreased by about 10%[68]. Bradycardia and reduced blood pressure on exertion[87] have also been observed.

D. *Treatment of Hypertension.* The clinical trials using guanethidine are summarized in Table 2. It is conspicuous that the oral dosages for guanethidine are smaller than that for bretylium, but it is not yet certain if this is due to a difference in extent of absorption or in relative potency. Most of the side-effects are largely the outcome of sympathetic nervous blockade, with predominance of the parasympathetic nervous system, particularly for the gastrointestinal tract.

6. CONCLUDING REMARKS. It is evident from the above discussion that a truly selective sympathetic nerve blocking agent is not yet available. Compound TM-10 has parasympathomimetic action, guanethidine has a transient sympathomimetic and ganglion blocking action, and bretylium has a transient sympathomimetic action. Undoubtedly, other similar agents will be discovered which will have a truly selective blockade confined to the sympathetic nervous system.

The exact place of sympathetic nerve blocking agents in the treatment of essential hypertension is not yet settled. There is a definite advantage over ganglion blocking agents in that the parasympathetic nervous system is spared. The regulation of the cardiovascular system appears to be shifted from a combined nervous-hormonal control to a combination of hormonal (adrenal medullary) and limited nervous control (vasodilators, but no cardioaccelerators or vasoconstrictors). This shift appears to be useful in reducing the blood pressure of hypertensive patients, but is such a shift useful in the long-term management of the disease? Will the usual complications be prevented and will the sympathetic nervous blocking agents arrest the progress of essential hypertension? Until such questions are answered, one will have to remain cautious in using the presently available sympathetic nervous blocking drugs.

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RADIATION THERAPY IN CARCINOMA OF THE ESOPHAGUS: A REVIEW

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IT is common knowledge that malignant disease in the esophagus is associated with a singularly poor prognosis, survival in untreated cases from the time of first diagnosis averaging less than 6 months. Treatment, with depressingly few exceptions, has not altered significantly this appalling outlook and if all patients are considered together, approximately 80% will have died within one year in spite of the best available treatment. It is, therefore, pertinent at this time briefly to review some of the published literature on the subject, in order to determine whether any improvement in the prognosis can be expected in the future with newer methods of treatment now available.

In any discussion of the treatment of carcinoma of the esophagus, it is necessary to keep in mind some of the salient features of the natural history, anatomy and pathology of this condition, particularly those which militate against the success of many methods of treatment advocated in the past.

NATURAL HISTORY. First, the disease most commonly occurs late in life, the average age of onset in most series being at least 60. Symptoms are of a chronic nature, and the patient has frequently lost weight and is dehydrated before he reports for medical advice, or before the physician is able correctly to diagnose his disease. Zuppinger[62] classified his 195 cases according to their clinical condition when first seen and found that 50% showed manifestations of cachexia with severe loss of weight and only 25% were in good general health.

Secondly, the commonest sites of occurrence of the disease are[36] 1) at the level of the 6th cervical vertebra; 2) at the crossing of the left main bronchus at the level of the 5th thoracic vertebra; 3) at the diaphragm. This would suggest that trauma may be a factor, for it is at these points that strictures most commonly occur following the ingestion of corrosive fluids. Furthermore, excessive consumption of alcohol has been sug-

gested[30,56] as an etiologic agent for the disease is considerably more common in those whose occupation involves exposure to alcohol, such as bar-men and waiters. The supposed association of the so-called Plummer-Vinson syndrome with the ingestion of hot tea is well known and hypopharyngeal carcinoma is particularly common in Scandinavian countries where this syndrome is prevalent. Similarly, carcinoma of the esophagus is common in China where hot drinks are frequently ingested[56]. Cardiospasm, with its associated long-standing dysphagia, and other chronic benign strictures have frequently been reported in association with carcinoma of the esophagus: it is possible that the continued irritation of retained food and liquids may contribute to malignant change. Such patients are undernourished from the chronic difficulty in swallowing and are poor candidates for extensive surgical or radiotherapeutic efforts.

Thirdly, diagnosis of this disease is seldom obtained early in its development because the symptoms are so insidious, for anatomical reasons which will shortly be considered. The size of the lesion is closely correlated with the extent of lymph node involvement[20].

ANATOMICAL CONSIDERATIONS. Several factors combine to explain the rapid downhill course of so many patients suffering from this disease.

1. The total thickness of the esophageal wall is only about 4 mm. and penetration by a carcinoma occurs early.
2. There is no serous coat to limit extension of the carcinoma once it has infiltrated into the muscle wall.
3. In close proximity to the esophagus are many vital structures such as the great vessels, the pericardium, the trachea, and the lungs, penetration into any of which may lead to rapid dissemination of the disease.
4. Direct extension into neighboring structures such as the recurrent laryngeal nerves or perforation into the mediastinum may occur early.
5. The blood supply is poor in the absence of a serous covering and this factor leads to poor healing after surgery. Any leakage from the suture wound is rapidly fatal from a spreading mediastinitis.
6. The lymphatic supply is very free and the vessels pass not only in an annular fashion but also lengthwise along the esophagus. Carcinoma in this area shows a remarkable propensity to spread for a considerable distance from the original growth although this may not be apparent clinically.

PATHOLOGY. In an analysis of 200 cases of carcinoma of the esophagus, Broders and Vinson[9] found that over 90% of squamous lesions were classified as Grade III or Grade IV and none of their cases were thought to be sufficiently differentiated to be placed in the category of Grade I. McGibbon[39] graded 448 cases and found that 56% were in Grades III and IV, 40% in Grade II and only 4% in Grade I. The chance of success of curative therapy is greatly reduced by this anaplasia.

The extensive lymphatic supply of the esophagus and the thinness of the wall, together with the longitudinal submucous spread of carcinoma imply early lymphatic involvement. Fleming[20] in an analysis of 76 cases found that in those in which the primary tumor measured more than 5.1 cm. in length, 88% had lymph node involvement or distant metastases, whereas if the lesion was less than 5.1 cm. in extent, only 50% of the patients showed such

involvement. Similarly, Sweet[54], out of 174 patients with carcinoma of the esophagus or cardia chosen as suitable for definitive surgery found that 50% had positive lymph nodes. It is evident that local therapy of any but the earliest lesions is doomed to failure in a high percentage of cases.

Choice of Treatment. 1. SURGERY. The purpose of this paper is to examine recent progress in the radiotherapy of this disease, but first it is necessary to assess the place of surgery in the management of carcinoma of the esophagus. When considering the relative merits of radiation therapy and surgery, the esophagus is usually subdivided into the anatomical areas of the cervical esophagus and of the upper, middle and lower thirds of the intrathoracic portion. These subdivisions are somewhat arbitrary and poorly defined especially at the upper and lower limits of the thoracic portion but treatment policy in these different areas varies because of their anatomical situation and pathology and they must be considered separately.

Cervical Esophagus. Attempts have been made to resect the cervical esophagus and, with adequate mobilization, to anastomose the stomach or small intestine to the pharynx and this is a practicable proposition although associated with a very high mortality. Wookey and others have devised operations for repair of the surgical defect in the pharynx and esophagus but none are entirely satisfactory, and in the opinion of Sweet[57], "In the neck it is practically impossible to approach the ideal radical extirpation necessary for a successful result from the point of view of cure." Radiation therapy of this area is relatively simple, has virtually no mortality and the morbidity is considerably less than that of extensive surgical procedures. The results of radiation treatment in the cervical esophagus are more satisfactory than in the other anatomical subdivisions and although the cure rate is still very low, there is a growing feeling that radical surgery should not be attempted in this area. In Table 1 are shown some of the reported results of treatment. From this it will be seen that one surgical series from Toronto gives a 5-year survival rate equivalent to that with radiation therapy elsewhere, but the mortality rate in this institution, where hypopharyngeal surgery was largely developed, was 15.6%[41].

TABLE 1.—RESULTS IN CERVICAL ESOPHAGUS

Author	*5-Year Survivors*	*Treatment*
Gynning[27]	2/7	200 KV Rotation
Jørgsholm[31]	8/109	200 KV
Lederman[34]	7/125	Teleradium
Mustard and Ibberson[41]	4/45	Surgery
Mustard and Ibberson[41]	1/55	250 KV
Sweet[55]	0/88	Surgery

Upper Esophagus. Much the same arguments apply in this area where the lymph node drainage may be upwards into the neck or downwards into the mediastinum rendering radical surgical extirpation virtually impossible. External therapy is again to be preferred, because the upper esophagus is not deeply situated and is, therefore, accessible to irradiation. Smithers[52] analyzed the results reported in 25 series which covered some 7000 cases. He found only two surgical survivors

for more than 5 years whereas no fewer than 33 treated with radiation therapy were free from disease at the end of that time. At the Royal Marsden Hospital itself, 6 out of 52 patients survived the arbitrary 5-year period.

Middle Third. The greatest diversity of surgical and radiotherapeutic opinion arises in this subdivision of the esophagus. Surgery is felt by many to be the treatment of choice and Sweet[57] reports a resectability rate of 66% in 182 cases of mid-thoracic lesions. Even in his experienced hands, however, the mortality from the surgical procedure is 25% if the lesion arises above the arch of the aorta and the survival rate at 5 years is only 6% *of those who survived the operation;* that is, only 5 cases. As will be seen later, results comparable with these are obtainable with various methods of radiation therapy without the hazards of surgery in terms of mortality and morbidity. Buschke[11], however, feels that in the constricting circular type of lesion which may remain localized in the esophagus for a relatively long period of time, surgery may be attempted if the patient is in good general condition.

The lymphatic drainage from the mid-esophagus is largely downwards towards the gastric plexus and if these nodes are involved, the results of surgery are still less than satisfactory, although an occasional patient may be salvaged. If only the lymph nodes in the immediate locality of the primary lesion are involved, it would seem at first sight that surgery should be the treatment of choice. Nevertheless, radiotherapy can be relied upon to sterilize such lymph nodes in many cases, as is seen when pre-operative irradiation is given, and the surgical mortality far outweighs the possible advantages of excision. Furthermore, a high percentage of cases are unresectable, because of direct invasion of neighboring structures and in such patients radiotherapy, with its ability to sterilize the primary lesion[58], might well sterilize also the local extension. If dissemination of disease has not already occurred, cure could theoretically result.

Lesions of the mid-thoracic region are commonest, representing 50% of 1,484 cases seen at Memorial Hospital between 1926 and 1957[59], as opposed to 25% in the lower esophagus and 25% in the upper and cervical esophagus combined. Other series, such as those at the Royal Marsden Hospital[51] and of McGibbon[39] confirm this distribution, and it is notable that in Sweet's series of surgical cases[55] there was a considerable preponderance of lower third lesions, 54% of the total. The terribly low present survival rate in this group represents the greatest challenge to progress in radiotherapy, if we assume that the limits of radical surgical extirpation have already been reached. It is in this area that we can particularly agree with Smithers[52] in saying "A careful selection is required by surgeons and radiotherapists together of the best possible treatment for each patient on the existing evidence, rather than on future hope, on unwarranted optimism, or unshakeable faith in personal skill."

Lower Third. There is no question that adenocarcinoma of the stomach secondarily involving the esophagus should be treated by excision wherever possible, for this is a practicable operation with an acceptable mortality and morbidity[40]. The same argument applies to squamous lesions arising in the proximity of the diaphragm where the line of excision does not reach to the level of the aortic arch. The lymph node drainage from the lower esophagus is almost entirely downwards to the gastric plexus and few

radiotherapists would feel that such an extensive area of hypersensitive tissue could be adequately irradiated with any apparatus at present available. The limitations of treatment are here imposed by the volume and the nature of the tissue irradiated rather than by any technical difficulties in achieving an adequate tumoricidal dose. Some successes with radiotherapy have been obtained in this area but Smithers' extensive analysis of 7000 cases[52] shows that only 13 radiotherapeutic survivors have been reported whereas 65 five-year survivors following surgery were found in the literature.

2. RADIATION THERAPY. In the view of many surgeons, radiation therapy is of value only in the palliation of carcinoma of the esophagus but successful curative surgery is also of recent date for in 1934 Souttar[53] stated, "It is only rarely that the question of radical removal of a carcinoma of the esophagus can even be raised, and in spite of a few brilliant results it seems to us most unlikely that it can ever become a practical method." In 1943, Smithers[50] could find only one case which had survived more than 5 years following resection, this being the famous patient of Torek, but by that time there had been several reports in the literature of patients successfully treated by radiation therapy. Since then, technical advances and interest in both surgery and radiation therapy have grown, and the number of radiotherapeutic successes has continued to increase as the surgical triumphs have begun to appear in the literature.

The object of radiotherapists since the early part of the century has been to develop techniques of radiation treatment which will sterilize the primary tumor without producing severe complications from damage to neighboring structures. In the past, it has not been possible to give adequate dosage to the necessary area but with newer techniques and apparatus these limitations no longer apply. There is no doubt that the primary disease can be sterilized in a very high percentage of cases by radiotherapy and, theoretically at least, such a patient should be cured of his disease provided that no metastases have occurred. The argument is often advanced by surgeons that patients with distant lymph nodal metastases cannot be cured by local radiation therapy but, in fact, the number of such patients cured by surgery is woefully low. For example, in Sweet's[57] series of patients surviving surgical extirpation, the 5-year survival rate in the lower esophagus is 32% if no nodes are found at operation but only 15% if all cases are considered. He, unfortunately, does not give the number of cases falling into each category nor the survival rate if nodes are found to be involved. If unsuccessful radiotherapeutic results are analyzed, it has been amply demonstrated that the great majority are due to the development of distant metastases and relatively few to persistence of primary tumor. Some failures have resulted from the complications of radiotherapy, and if these cases are obviated with more accurate dosimetry and localization of therapy, there would appear to be no reason why the results of well planned radiation therapy should not be as good as those of surgery. If one takes into account the high mortality of surgical excision, the results should, in fact, be better. In some areas of the esophagus this is already true, as Buschke[11], analyzing representative figures from surgeons and radiotherapists, showed. By reference to Table 2, it will be seen that in the thoracic esophagus surgical excision resulted in a 5-year survival rate of 2.5% of all patients seen, whereas 3.6% of those seen

in a radiotherapy department, presumably often because they were inoperable, survived that period. It would appear possible that many of those who are "cured" by surgery especially in mid-thoracic lesions might equally well have been successfully treated by radiation therapy and some who died of the operation might have survived.

TABLE 2.—COMPARISON OF SURGICAL AND RADIOTHERAPEUTIC 5-YEAR RESULTS IN MID-ESOPHAGUS (AFTER BUSCHKE[11])

	Surgery	*Radiotherapy*
All cases seen	2.5%	3.6%
Treatment completed	3.3%	6.2%

TECHNIQUES OF RADIOTHERAPY. Chronologically, the first attempts at radical treatment of malignant disease of the esophagus with ionizing radiations were made with intracavitary sources of radium. Subsequently, external Roentgen-ray therapy at 190 to 250 KV with various techniques, teleradium, telecobalt and supervoltage apparatus from 800 KV to the 31 Mev betatron, have been employed. Consideration will now be given to these techniques individually, to their advantages and disadvantages, and to the results obtained therefrom.

Intracavitary Radiation. From the earliest years of the usage of radium in cancer therapy, attempts have been made to introduce a source of radiation into the lumen of the esophagus to lie at the level of the carcinoma and thus to irradiate the tumor from within. Guisez[25] was an early proponent of radium used in this fashion. The technique was elaborated with more accurate dosimetry by Lederman and Clarkson[35], and Watson and Goodner[59] also used intraluminal radium in a small number of cases. A combination of intracavitary radium tandem and external therapy was used by Zuppinger[62], by Watson and Goodner[59] and others, but resulted in serious complications, particularly perforation.

Other methods of applying intracavitary radiation include the attachment of radon seeds to an indwelling tube of the Souttar type[14,53], and radioactive tantalum wire in a Souttar Tube[7]. It has also been suggested that a tight spiral tubing filled with liquid radioactive isotope could be passed into the esophagus at the desired level.

Finally, the direct implantation of malignant tumors of the esophagus has been attempted[17,42,53] and growths exposed at surgery have been implanted with radon[23,29,59]. At the Royal Marsden Hospital, radioactive gold grains are occasionally implanted into carcinoma of the esophagus in inoperable lesions with the aim of superficial destruction of tumor and consequent increase in the lumen of the esophagus[35].

The limitations of this approach to the problem are obvious from consideration of the anatomical and pathological features of the disease. First, an adequate dosage of radiation is obtainable only to the most superficial layers, unless the risk of necrosis of the mucosa from over-dosage is taken in order to increase the dosage at a depth. It has been calculated that 5000 rads delivered at 1 cm. from the surface of a radium applicator falls to 2500 rads at 1.6 cm. and this would be totally inadequate for a tumor which had infiltrated through the esophageal wall. On the other hand, to increase the mucosal dose above this level would lead to almost certain necrosis. Secondly, the propensity of esophageal carcinoma to spread submucosally makes it necessary to treat an area very wide of the obvious primary lesion and local radiotherapy to the tumor itself will not sterilize the intramural spread. Thirdly, the rapidity

with which the radiation is given may, in a highly radiosensitive tumor, lead to rapid necrosis and in an organ with a wall as thin as that of the esophagus, perforation may readily ensue. An added disadvantage of the implantation technique is the difficulty of securing an even distribution of the radiation, and once the seeds have been implanted they cannot readily be removed in order to correct areas of high or low dosage.

In spite of these disadvantages several long term favorable results have been reported[1,25] with this type of radiation in the past. Although it is not to be recommended when other more effective techniques are available, there is still a place for this approach. In patients who are otherwise untreatable or who have already had the maximum external radiation or surgery and yet still have disease localized to a small area, the insertion of a radium catheter or radioactive gold grains may lead to superficial destruction of the obstructing lesion and enable the patient to swallow more readily.

Teleradium. Radiation units containing relatively large quantities of radium were extensively employed for the treatment of squamous carcinomas but the scarcity of radium and the serious problems of protection limited the quantity available in any one unit. The output of radiation from these units was, therefore, relatively low and in order to give the required dosage in a reasonable length of time the treatment distance seldom exceeded 10 cm. For physical reasons, this reduces the percentage depth dose of radiation very considerably, and in an organ as deep seated as is the esophagus, the limitations of such treatment were readily foreseeable. However, in the cervical esophagus an adequate dosage was obtainable without serious skin reactions and in this area considerable experience was gained[34,35]. With the development of supervoltage Roentgen-ray and telecobalt machines, teleradium therapy in the esophagus has fallen into abeyance.

Conventional Roentgen-ray Therapy. Because of its deep situation within the thorax and the strictly limited dosage attainable at a depth with Roentgen-ray machines of early design, external radiation of carcinoma of the esophagus was not felt to be practical. However, with the technical advance of equipment generating Roentgen-rays at 190 KV and upwards and by use of multiple portals of entry, it was found possible to deliver a respectable dose at the level of the tumor, and in the early 1930's, reports of successful primary healing of esophageal lesions were made from many centers in America and in Europe. Initially, the doses calculated at the site of the tumor were low by present standards but with increasing elaboration of technique and more accurate beam direction of the treatment fields, tumor doses approaching 6000 r were obtainable.

In a review[21] of some 800 cases published before 1947, a 5-year survival of about 1.2% of the total was reported. Table 3 summarizes a further series of reports of cases treated at from 190 to 250 KV in which an overall 5-year survival of 1.4% of 897 cases was obtained. In this table are included some cases of cervical esophageal lesions which, as already stated, have a somewhat better prognosis than lesions within the thorax.

Rotation Therapy at Conventional Voltage. Many techniques required the use of very many portals of entry (Roberts[48] used up to 26 ports 1 cm. apart). The next rational development from this multiplicity of treatment fields was that of rotating the patient within the beam of radiation, Scandinavian authors being pioneers in this

method[26,27,33,43,44,45,46]. It was appreciated that the width of the treatment field should be reduced as far as possible so that the column of tissue irradiated to high dosage should not extend to include the vital structures in close proximity to the esophagus.

TABLE 3.—SOME 5-YEAR RESULTS WITH FIXED FIELD CONVENTIONAL VOLTAGE RADIATION

Author	*Results*	*Method*
Baclesse[4]	1/128	200 KV
Köhler[32]	2/296	190 KV
Smithers[52]	7/198	400 KV
Watson and Goodner[59]	3/275	200–250 KV
Total	13/897 = 1.4%	
Compare Fleming[21]	10/800 = 1.2%	

The difficulty of insuring that the area in question was always within the treated field was overcome by the use of continuous fluoroscopy of the esophagus which had been filled previously with a radiopaque material. The exposure of personnel watching the fluoroscopic screen was not inconsiderable. The fluoroscopic image was, therefore, reflected in a mirror so that the control area was not in the direct beam of radiation. The operator could then follow the image reflected in the mirror as the patient rotated and could alter the shape and position of the treatment beam by remote control. When image intensification became practicable, it was found possible to visualize the lesion as a televised image throughout the treatment time[18]. In this way, the lesion was kept within the area of treatment throughout the rotation time and the field size kept to a minimum of not more than 5 cm. As a consequence, radiation fibrosis of normal tissues was not encountered.

One serious disadvantage of rotation treatment is that, in lesions encroaching upon the lower third of the esophagus, the anterior angulation of the part precludes the use of a long treatment field of minimum width, for in the lateral position of the patient the lower part of the lesion will of necessity be outside the field. Alternatively, one is forced to use a wider column of radiation to include this angulation which is equally unsatisfactory. In carcinoma of the mid-esophagus, however, the results of rotation therapy at 250 KV show considerable improvement over those obtained with stationary field treatment and some of the results obtained are summarized in Table 4.

Supervoltage Therapy. In 1943, Watson and Urban[60] reported satisfactory palliation in 63 cases of carcinoma of the esophagus treated by one million volt Roentgen-ray therapy.

TABLE 4.—SOME 5-YEAR RESULTS WITH 190–250 KV ROTATION THERAPY

Author	*Results*
Ebenius[19]	9/119
Krebs[33]	1/65
Nielsen[44]	9/423
Watson and Goodner[59]	1/56
Total	20/663 = 3.0%

Since that time, statistical results have been reported from many centers using radiations from 800 KV to 31 Mev betatron beams[2,3,6,7,8,11,12,13,15,16,21,28,37,47,58,59]. Much of this equipment has been in clinical use for a relatively short period of time and it is not possible to compare the long-term results of such treatment with those obtained with less penetrating radiations. An interesting comparison, however, is made by Bernard and his associates[6] who found that in 95 cases treated at 200 KV, the average survival was 5.5 months whereas in 46 patients treated with the 22 Mev betatron the average survival was 8.8 months. Furthermore, 14 out of 93 (15%) of the earliest series

survived more than 10 months, whereas 17 out of 44 (38%) of the betatron series survived that length of time. Buschke and Cantril[12] have had extensive experience with an 800 KV machine and in 1953, reported three 5-year survivors out of 14 patients who had received a full course of therapy. Fifteen others did not complete their treatment.* The percentage of patients surviving relatively long periods after treatment appears, therefore, to show some improvement with the use of supervoltage but again, the only patients who can be expected to be benefited for long are those whose disease has not yet spread beyond the confines of the esophagus. It should be reiterated that an area well wide of the obvious primary lesion must be treated in order to control potential longitudinal spread of disease if radiotherapy is to be the only method of treatment. Fortunately, with very high voltage equipment such as the linear accelerator and betatron, it is theoretically possible to irradiate the whole esophagus from thoracic inlet to diaphragm in one continuous field, and, as it is not necessary to use lateral portals to increase the depth dose, the anterior angulation of the lower esophagus does not preclude the use of such a long field. Techniques such as these are being developed and are in use in at least one institution[61].

Combined Therapy. As in so many other areas, where the results of neither surgery nor radiotherapy in the treatment of malignant disease are satisfactory, the possibility of combining the two modalities has been suggested. Unfortunately, the difference of opinion between surgeons and radiotherapists as to whether the radiation should precede or follow radical surgery has not been resolved. From the point of view of the radiotherapist there is no question that pre-operative radiation is very greatly to be preferred, for one is dealing with untreated malignant disease in tissue which has not been disturbed and in which body resistance has not been reduced by operative interference. Postoperative radiation is perforce administered to a fresh suture line, to tissue with a compromised blood supply and, in many instances, to small intestinal or gastric epithelium which is notoriously sensitive to high doses of radiation. The proponents of postoperative irradiation, particularly at the Lahey Clinic[2,3,8], do not mention this disadvantage in their papers and do not appear to have had the experience of an increase in breakdown at the suture lines nor of difficulty in delivering an adequate radiation dosage because of the presence of bowel. Others[10,18], however, report that the presence of small intestine in the treatment field in a patient whose lesion has been bypassed prior to the radiation limits the dosage which can be delivered.

Pre-operative radiation is not acceptable to many surgeons on the grounds that it is said to lead frequently to poor healing. For the esophagus, this would, of course, lead to necrotic breakdown and mediastinitis. If the timing of the operation is chosen properly, however, experience at other sites has shown that this is not necessarily true. In several cases reported[12,15,16,18], pre-operative irradiation of the esophagus followed by resection of the involved segment has not been followed by serious complications. Cliffton advocated that irradiation be limited to

*ADDENDUM: Dr. Ivan Smith (personal communication, 1961) reports that of 31 cases receiving radical treatment with telecobalt between 1952 and 1956, six are alive between 55 and 98 months after treatment and one died 72 months after treatment with what was presumed to be recurrent disease in the abdomen.

the area shown radiologically to be involved, and that subsequent excision be wide of the irradiated tissue, especially if a total esophagectomy be performed. In his series of 11 patients[16] who had both irradiation and surgery, no difficulties were encountered and no breakdown of the scar occurred. Full pathological examination of 6 of the specimens revealed either no tumor, or tumor limited to the mucosa and submucosa, and in none was there extension into the mediastinum or active tumor in any of the local lymph nodes. Because of the finding of multiple sites of carcinoma *in situ* in one patient, he recommends total removal of the esophagus in all cases. Mallet and Decker[38] suggested pre-operative rotation therapy at 190 KV prior to surgery, but this was on the basis of their experience with radiation alone and no further reports have apparently been made of any results obtained.

Personal experience with pre-operative irradiation is limited to 2 cases. In one, a stricture resulting from radiation therapy was excised, no tumor was found and no difficulties were encountered at operation. In the other case, a lesion of the thoracic esophagus was successfully irradiated and one year later a second carcinoma of the cardia was resected. Even though the anastomosis was within the previously irradiated esophagus, no complications of this combined therapy were noted.

The optimum time for resection following pre-operative irradiation would appear to be not less than 6 weeks and not more than 3 months after the completion of the radiation. Surgery prior to the 6-week interval may be made more difficult by the vascularity of the part induced by the radiation therapy. On the other hand, if surgery is delayed more than a few months, the fibrosis and impaired blood supply consequent upon the radiation therapy may lead to difficulty in healing and possible breakdown of the anastomosis.

In any discussion of combined therapy, the question of *chemotherapy* must be considered. Squamous carcinoma of the esophagus is an epidermoid tumor and to date no chemotherapeutic agent has been shown to be of significant value in any epidermoid carcinoma. It is difficult to believe, therefore, that the administration of toxic drugs in patients already debilitated by their disease is justified except on an experimental basis. Until the day arrives when an agent known to be effective in this disease is available, cytotoxic drugs should be withheld and and not used either alone or in combination with more established methods of therapy. The great majority of patients with carcinoma of the esophagus cannot be cured by surgery or radiotherapy or by a combination of the two, and palliation of their symptoms is all that can be achieved. Many methods of successful palliation are available to us and if well chosen will not add to the patient's discomfort although they may not significantly prolong his life. One must guard against therapeutic enthusiasm as a result of which the patient may be made more uncomfortable for the few weeks or months remaining to him.

RADIATION TOLERANCE. Seaman and Ackerman[49] studied several necropsies on patients who had been treated for carcinoma of the lung by betatron irradiation and noted marked changes in the normal esophagus which was within the treatment area. From a study of these cases they came to the conclusion that the tolerance of the normal esophageal wall to radiation of this energy was in the region of 6000 rads given in 6 weeks. It is interesting, in studying the literature, that the optimum dosage for esophageal carcinoma advocated by most authors is

within this range, clinical experience correlating well with the pathological findings. Slightly higher doses at a somewhat more rapid rate were given by Gynning with 200 KV rotation therapy[26] and by Lott and Smith with cobalt therapy[37]. These latter authors administered 6000 to 6500 rads in 4 to 4½ weeks.

From a study of necropsy cases the effects of over-dosage are often apparent in patients who develop fistulas from necrosis and in those who have marked stenosis following irradiation. Such complications become more frequent as the tumor dose is increased or if treatment is given more rapidly than 1000 r per week. If these facts are borne in mind, it is felt that the incidence of fatal complications from radiation therapy will be reduced, without a significant reduction in the number of lesions which are sterilized. It is to be recommended, therefore, that the total tumor dose should not exceed 6000 rads in 6 weeks or its equivalent in a longer or shorter time. At this treatment rate also it is to be expected that the healing of the esophagus will keep pace with the destruction of the tumor, thus reducing the incidence of perforation during treatment.

ANCILLARY THERAPY. Certain minor surgical procedures may assist the radiotherapist in carrying out a palliative or curative course of irradiation. These are in essence three: 1) gastrostomy; 2) feeding tube; 3) Souttar tube.

1. *Gastrostomy.* Gastrostomy alone seldom relieves the symptoms of dysphagia for, although the patient may be enabled to take nourishment, he still cannot swallow salivary secretions and he remains equally as uncomfortable as he was prior to the operation. There is little evidence to suggest that a gastrostomy *per se* will add to a patient's life span and such a procedure would not appear to be justified if the esophagus is completely obstructed. Bernard[6] suggests that a gastrostomy should be performed prior to the onset of severe symptoms of dehydration during a course of radiation therapy if the patient is not tolerating the treatment well. It is in such a situation that this operation may have a place. Similarly, in those patients who develop tight strictures following radiation therapy, it may be of great benefit to allow nourishment to be given through a gastrostomy tube provided that there is sufficient esophageal lumen that the patient can swallow his own secretions. It is occasionally found that such strictures cannot be kept sufficiently dilated for normal swallowing to occur. Watson and Urban[60], in their series of palliative one million volt therapy, state that gastrostomy was performed in most of their patients prior to the treatment: but the value of this procedure cannot be assessed from the results reported and it is questionable whether this is the treatment of choice.

2. *Feeding tube.* In patients whose lesion has become so large that the lumen of the esophagus is almost completely obstructed, great benefit may ensue if a small feeding tube can be passed transnasally through the obstruction. The patient may then be fed through the tube during his course of radiation therapy. Although the difficulty in swallowing saliva is still present, the feeding tube will frequently keep sufficient lumen in the esophagus that liquids may pass by without difficulty. This method of sustaining the patient's nutrition is, therefore, to be preferred to a gastrostomy in most cases.

3. *Souttar tube.* Many varieties of self-retaining esophageal tubes have been designed and that described by Souttar is used only as a representative example in this discussion. From the surgical standpoint, the passage of a

metallic or plastic tube through an esophagus, already diseased by carcinoma and in which the lumen is frequently obscured, is fraught with danger; for false passages may be created, or undue pressure on the tube may lead to perforation of the weakened esophageal wall. Such a tube placed in the esophagus prior to radiation therapy is also inconvenient from the standpoint of the radiation therapist. First, the possibility of secondary radiation from a metallic tube at the level of the esophageal constriction may lead to a rapid high dose effect with destruction of tumor and resulting perforation before the desired healing has occurred. Secondly, as the lesion shrinks, the tube becomes loosened and will rapidly pass through into the lower bowel. Such a feeding tube is, therefore, not to be recommended preliminary to a full course of radiation. It is of significant value, however, in the retention of an esophageal lumen if a stricture at the site of a treated carcinoma appears to be imminent. Unfortunately, in such cases it is seldom possible to pass a tube of adequate bore.

PALLIATIVE THERAPY. Only a small percentage of fortunate patients have survived for long periods following radical treatment and one should surely continue giving such treatment whenever there is a possibility of a satisfactory outcome. Certain clinical findings, however, herald an early termination and when these are present, one must weigh the balance of benefit to the patient from a course of treatment against the proportion of the remaining weeks of life left to him which are perforce spent in daily attendance to undergo this treatment. It is, for instance, surely not justifiable to embark upon a course of treatment lasting some 6 weeks when the expectation of life is only 2 or 3 months and when a shorter course of treatment may relieve the symptoms and enable the patient to succumb in relative comfort to his disease. In the presence of distant metastases to the supraclavicular nodes or to the liver, the sterilization of the primary lesion will allow the patient to swallow but will not prolong his life. Similarly, obvious involvement of a long segment of the esophagus is usually associated with spread to the lymph nodes and the chances of lasting benefit are minimal. In such instances, the aim of radiation therapy should be to relieve the symptoms as rapidly as possible without producing new symptoms due to the treatment. Attempts have been made to deliver a dose of 1500 r to the esophagus in one or two treatment days[22,23] but such a procedure in a patient already debilitated will almost invariably produce considerable side effects from the treatment. On the other hand, it is frequently possible to increase the lumen of an esophageal carcinoma by the administration of a tumor dose of 2500 r in 10 days. Such treatment can be given without significant discomfort to the patient and with great symptomatic relief both physical and mental, however temporary this may be. The presence of supraclavicular nodes or other distant metastases which are asymptomatic does not justify therapy, for in the absence of symptoms, such treatment can benefit no one but the radiotherapist as a demonstration of his ability to reduce the size of palpable nodes. The presence of bony metastases or of other symptomatic lesions, of course, calls for active treatment to be given in as short a time as is convenient.

CONTRAINDICATIONS TO RADIATION THERAPY. Papillon and Goyon[47] tabulate certain findings which they consider to be absolute contraindications to radiation therapy whether it be given

by external therapy or by internal sources and whether for radical or palliative effect. Some of their contraindications, such as the presence of a primary lesion more than 12 cm. in length and the presence of distant metastases, do not necessarily preclude external therapy in palliative doses but the presence of other manifestations should certainly make one hesitate before administering any irradiation. These are: 1) fistula; 2) infection; 3) cachexia.

1. *Fistula.* Radiation therapy of a lesion which has a demonstrable communication with the tracheobronchial tree can lead to nothing but disaster. In such instances the communication will be significantly enlarged as the tumor mass responds to the radiation therapy with consequent increase of the channel by which infected material can reach the lungs. In such instances, an endoesophageal tube is of very great benefit as it will frequently seal off the communication sufficiently that nourishment will pass normally through the esophagus. A gastrostomy alone does not prevent the inhalation of salivary secretions and is seldom indicated. If a soft tissue swelling is visible surrounding the esophageal carcinoma but no fistula can be demonstrated radiologically, Roentgen-ray therapy may still be of considerable benefit to the patient. This soft tissue mass may represent extension into the mediastinum without perforation, but the presence of a primary bronchogenic carcinoma secondarily involving the esophagus must, of course, be excluded.

2. *Infection.* In the presence of marked inflammatory changes in the esophagus and mediastinum, irradiation may well increase the severity of the infection and its spread. Prior to active treatment, therefore, such patients should receive adequate antibiotic therapy and not until the infection has subsided should the radiation treatment be given. Occasionally, a patient with active pulmonary tuberculosis may develop carcinoma of the esophagus. In such cases, radiation therapy may be given but adequate dosage with antituberculous drugs must be insured, lest exacerbation of the infection be produced.

3. *Cachexia.* Some patients suffering from carcinoma of the esophagus, present themselves with no acute symptoms of dysphagia but with generalized cachexia, and no evidence of symptomatic metastases. The value of local therapy to the esophageal lesion is questionable if it is asymptomatic. In such instances, one would probably recommend a feeding tube or a gastrostomy to maintain the patient's nourishment, although this may not contribute significantly to lengthening his life.

Discussion. Carcinoma of the esophagus remains a fatal disease in the vast majority of cases, and in spite of great efforts toward improving the prognosis, little significant progress has been made. Success with present-day methods of surgery and radiotherapy must be limited to those whose disease is diagnosed before widespread metastases have occurred. Over the years there has been a very slight improvement in the survival rate and with consideration of some of the important anatomical and pathological factors, modern methods of treatment should be able to achieve still better results. In particular, instances of local recurrence at the site of a primary lesion previously sterilized by radiation therapy or removed by surgery should, in theory, be avoidable. In the mid-thoracic region, the commonest site for carcinoma of the esophagus and the one with the least favorable prognosis, the most radical of surgeons cannot report more than a small percentage of survivors,

even in selected cases, and have to admit that a large number of their patients succumb to the operation. Similarly, radiotherapists report only the occasional success in this area. The possibilities of combining the two modalities, giving a full course of radiation therapy to the whole esophagus and then performing radical surgery at a later date, have not been sufficiently explored, but the initial results are somewhat encouraging. The reverse combination of surgery and postoperative radiation is less satisfactory.

Lesions of the lower esophagus, being preponderantly adenocarcinomas, are less sensitive to the effects of ionizing radiations. Surgical excision with a good margin may not infrequently lead to acceptable mortality and cure rates, whereas results of radiation therapy are less well defined. Surgery is undoubtedly the treatment of choice in this area.

On the contrary, in lesions of the cervical and upper esophagus, it is more than questionable whether surgical excision with the great morbidity associated with operations in this area is justified by the small number of survivors. External radiation therapy with modern techniques and apparatus can be delivered with adequate tumoricidal doses to the primary lesion and to the potentially involved lymph nodes. In some institutions, reasonably satisfactory survival rates have been reported.

There remains the place of radiation therapy in the palliation of symptoms and it is in this field that most experience is gained, for a large proportion of the patients when first seen already have evidence of spread beyond the limits of even theoretical curability and only palliation can be offered. The results of such treatment are reasonably satisfactory with external radiation given in as short a time as is practicable and with appropriate minor surgical procedures when indicated. The radiotherapist must, however, judge each patient individually and insure that treatment is given only for betterment of the patient, for in certain instances exacerbation of the symptoms can be produced. We must insure that, to borrow a quotation from the Odyssey from Dr. Ff. Roberts[48], our patients do not

". . . waste piece-meal day by day,
With slow disease and withering undelight."

Summary. Some of the more important aspects of the natural history, anatomy and pathology of carcinoma of the esophagus are discussed in relation to treatment of this disease.

2. The literature is briefly reviewed and it is concluded that:

a. Lesions of the upper esophagus are best treated by radiation therapy.

b. In the lower esophagus surgery is the treatment of choice.

c. In the mid-thoracic region there is considerable variance of opinion.

3. The argument is advanced that radiation therapy is to be preferred to surgery in mid-thoracic lesions but that a combination of pre-operative radiation and excisional surgery should be given an extended trial in selected cases.

4. The development of radiotherapeutic techniques through the years is reviewed. It is pointed out that with modern apparatus it is possible to treat the whole of the esophagus to a tumoricidal dose without ill effect on the normal tissues.

5. An estimate is made of the probable tolerance of the esophagus to radiation, above which dose complications appear likely to ensue.

6. Indications for, and methods of, palliative radiation therapy are discussed and factors contraindicating any active radiation therapy are mentioned.

7. A statistical analysis of results obtained with different methods of treatment reported in the literature is presented. From these it is seen that the results of radiation therapy in intrathoracic lesions appear to be improving with the development of more penetrating radiations. Similarly, the results of surgery have improved but more radical surgery yet is associated with a very high mortality in lesions at or above the level of the aortic notch. It would appear that the limits of surgical extirpation have been reached.

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SEMINAR ON CARDIOVASCULAR PHYSIOLOGY

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Effect of Status of Circulation on Changes in Thoracic Aorta Blood Flow in Man During Valsalva Maneuver. NEWTON C. BIRKHEAD, M.D. (Division of Research, Lankenau Hospital, Philadelphia, Pennsylvania), and EARL H. WOOD, M.D. (Section of Physiology, Mayo Clinic, Rochester, Minnesota). Characteristic responses to the Valsalva maneuver have been reported to occur in valvular heart disease, congestive heart failure and following dorsolumbar sympathectomy. The majority of such observations are based on interpretation of central and peripheral arterial and venous pressure changes, since data on blood flow changes under such circumstances are scarce. To study further the mechanisms of these responses, thoracic aorta blood flow was measured continuously by the constant-rate injection indicator dilution technique in 6 healthy men during the Valsalva maneuver (40 mm. Hg airway pressure for 15 seconds) in (1) 10° head-up body tilt, (2) 10° head-down body tilt, (3) in the 10° head-up tilt with a g-3A antiblackout suit inflated to 150 mm. Hg and (4) in the 10° head-up tilt following partial ganglionic blockade with intravenous tetraethylammonium chloride (5 mg. per kg.). Right atrial, aortic and peripheral arterial pressures were recorded simultaneously with aortic flow. Mean control thoracic aorta flow was 3.3, 3.5, 2.8, and 3.4 per min. and minimal values during the maneuver were 41.1, 62.6, 61.4, and 23.1% of control values under conditions 1, 2, 3, and 4 above respectively. The increase in central intravascular pressures especially on the venous side of the circulation were believed responsible for the beneficial effect of the head-down tilt and lower body pressurization. The detrimental effects of ganglion blockade were associated with interference with compensatory reactions in both the arterial and venous side of the circulation. Inter and intra individual variations in the response of the circulation to the Valsalva maneuver were found to be based in part on the heart rate and pressure flow relationships existing just prior to the beginning of the maneuver.

Differential Monophasic Action Potentials of the Atrium and Ventricle of the Dog's Heart. JOSEPH R. DIPALMA, M.D. (Department of Pharmacology, Hahnemann Medical College and Hospital, Philadelphia). In order to make a comparison between the monophasic action currents of the atrium and ventricle the following experiments were performed. The chest of dogs anesthetized with Dial-Urethane was opened under artificial respiration and the heart exposed. Special clip electrodes with a tip diameter of 10 thousands of an inch steel wire were attached to selected sites on and in the heart. These were S.A. node vs. Right Ventricle, A.V. node vs. Right Ventricle, Right Atrial Appendage vs. Right Ventricle, A.V. node vs. S.A. node, A.V. node vs. Bundle of His and A.V. node vs. the Intraventricular septum. Since a

differential amplifier was used and each electrode had no ground reference point the action potential recorded was of one area of the heart versus another in the positions noted above. In the records obtained of the S.A. node vs. the Right Ventricle the potential at the S.A. node showed little initial negativity with a gradually increasing negative potential towards the end of electrical activity. With excitation of the ventricle there was a large monophasic negative action potential of characteristic shape. When the A.V. node or any other area of the atrium, such as the atrial appendage, was studied the atrial action potential was always opposite in direction to the ventricular action potential. This could be interpreted to mean simply that at the time the atrium was excited it was negative in sign to the ventricle and vice versa. However, direction of excitation or wavespread was also a factor for when the same electrode positions were studied under conditions of retrograde conduction the atrial and ventricular potentials were in the same direction. When the electrodes were placed on the S.A. node vs. the A.V. node only an atrial action potential occurred. The same held true for the A.V. node vs. the Bundle of His. Movement of the lower electrode to the Intraventricular Septum gave a typical A.V. node Right Ventricle pattern. This substantiates the view that ventricular excitation does not occur until the cardiac impulse has traversed the Bundle of His. One overall deduction permitted by observations from all the electrode positions is the relative electronegativity of the ventricle as compared to the atrium. Thus the ventricle serves as a natural "sink," for the impulse of the heart. The electrodes and methods described are of great usefulness in the experimental study of myocardial ischemia and the analysis of the actions of drugs on the heart.

Mechanisms of Quinidine Action on the Circulation and Therapeutic Reversal of Toxic Effects. H. L. CONN, JR., M.D., R. J. LUCHI, M.D., AND J. H. HELWIG, JR., M.D. Despite wide recognition of the electro-mechanical cardiac depressant and vasodilating effects of quinidine, the basic mechanisms responsible are obscure. Equally obscure are the mechanisms responsible by which the commonly used therapeutic agents reverse these effects. Even the usefulness of some recommended therapeutic agents is in doubt. Our data and those of others, derived from perfused heart experiments, have indicated that cardiac depression may in great part be due to derangement in cation movements and distribution. Subsequent *in vitro* dialysis studies of quinidine-protein binding led us to suggest that there is a specific binding at the cell surface between quinidine, cell membrane, and adjacent water. As a result of this special molecular orientation of quinidine, cell permeability is altered, ionic derangements ensue, and demonstrable electrocardiographic changes develop. Reduced muscle contractile rate may result from a similar series of events. In corroboration, no cardiac electro-mechanical alterations were seen in intact dogs as a result of infusing a quinidine homolog (4 hydroxymethyl 6 methoxy quinoline) which lacks the necessary configuration to form a protein-water bridge. This drug did produce vasodilatation, an action which is thereby considered due to the quinoline ring fraction of quinidine operating at a non-membrane site. The vasodilatation was shown to lead to hypotension on the basis of direct vascular drug action in competition with pressor amines. A satisfactory explanation is thereby provided for the known efficacy of pressor amines in treating quinidine toxicity. The conclusion was further corroborated in our

reversion of toxic effects by adequate dosage of hypertensin and levarterenol. Previous studies had suggested that changes in ionic composition, especially through sodium lactate administration, would reverse quinidine effects. We studied both changes in hydrogen and calcium ion concentration. Versene infusions alone partially reversed the hemodynamic and electrocardiographic abnormalities, and nearly complete reversion was obtained by the combination of versene and pressor amines. In contrast, both decreasing pH by acid administration and increasing pH by administration of sodium lactate either failed to exert a favorable response or actually aggravated the toxic state.

BOOK REVIEWS AND NOTICES

OCCUPATIONAL DISEASES AND INDUSTRIAL MEDICINE. By RUTHERFORD T. JOHNSTONE, M.D., University of California at Los Angeles AND SEWARD E. MILLER, M.D., University of Michigan. Pp. 482; 71 ills. Philadelphia and London: W. B. Saunders Company, 1960. Price, $12.00.

THE authors, obviously well qualified in the field of industrial medicine, have compiled an excellent text on this subject. The book is in two parts.

Part one deals with the practice of industrial medicine. It presents an up-to-date concept of industrial medicine as it should be practiced today. There are chapters dealing with the much needed aspects of industrial medicine and insurance and man's adaptation to industrial life.

Part two is concerned with the occupational diseases including those illnesses arising from industry's new technologies and is presented in a very comprehensive manner.

An appendix is devoted to threshold limit values for 1960 and there is an excellent glossary of some industrial terms.

The book is highly recommended for all those who participate in the practice of industrial medicine. J.M.

THE CENTRAL NERVOUS SYSTEM AND BEHAVIOR. Edited by MARY A. B. BRAZIER, PH.D., Neurophysiological Laboratory, Massachusetts General Hospital, Boston. Pp. 358; 89 ills. New York: Josiah Macy, Jr. Foundation and the National Science Foundation, 1959. Price, $4.75.

THIS volume contains the proceedings of the Second Conference on The Central Nervous System and Behavior which was held in February, 1959. Among the principal topics discussed were: "The Limbic System with Respect to Two Basic Life Principles;" "The Hippocampus and Higher Nervous Activity;" "Reversible Decortication and Behavior;" and "Electroencephalographic Studies in Conditional Reflex Formation in Man." In addition, there is an interesting presentation concerning an International Colloquium on EEG which convened in Moscow in October, 1958.

Workers in neurophysiology and the behavioral sciences will find in these pages much valuable and informative discussion. C.Z.

MEANING AND METHODS OF DIAGNOSIS IN CLINICAL PSYCHIATRY. By THOMAS A. LOFTUS, M.D., Associate Professor of Clinical Psychiatry, The Jefferson Medical College, Philadelphia, Pennsylvania. Pp. 169. Philadelphia: Lea & Febiger, 1960. Price, $5.00.

THE author draws upon a wealth of clinical experience to organize psychiatric knowledge into a very comprehensive compendium designed to clarify the problem of diagnosis. In the early portion of the book, he emphasizes the fact that diagnosis should be considered in terms of "reaction types." This would be considered a "working diagnosis for the treating physician." He openly discusses the problem of diagnosis related to ". . . social, legal, economic or cultural factors . . ." It is his opinion that introducing these factors into diagnosis only brings confusion to the issue. He recognizes the need for it and solves the conflict by making use of two diagnoses, one called a "referential diagnosis" dealing with the concept of the reaction-type, and the other called a "statistical diagnosis" dealing with a label that may be used for "disposition." It is to the credit of the author that he brings this problem out in such bold relief.

The organization of the book is to be commended with case material being used to illustrate various parts of the psychiatric history and examination. A very unique feature is the presence of a section dealing with psychiatric histories, followed by questions based on the histories. This is a very interesting innovation in a textbook and should be of great value to the beginning student. K.C.

ANNUAL REVIEW OF MEDICINE, VOL. 11, 1960. Edited by DAVID A. RYTAND, M.D., AND WILLIAM P. CREGER, M.D. Pp. 453; 8 ills. Palo Alto, Calif.: Annual Reviews, Inc., 1960. Price, $7.00.

THE latest in this series of Annual Reviews of Medicine follows the newer trend of presenting more concentrated discussions of relatively limited fields. This permits the inclusion of a few more topics than before, which will be a welcome change. Among the chapters of this volume are: 'Gastrointestinal Disease: Portal Hyptertension" by C. G. Child, III; "Cardiovascular Disease: Pulmonary Hypertension" by A. A. Liebow; "Hormonal Control of Pigmentation" by A. B. Lerner;

"Biochemical Changes in the Dermatitides" by A. C. Curtis and W. D. Block; "Delayed Sensitivity and Homograft Sensitivity" by H. S. Lawrence; "The L.E. Cell Phenomenon" by H. R. Holman; "Pediatrics: Congenital Enzyme Defects" by J. W. Gerrard and A. M. Marko; and "Special Therapeutics: Physiology of Diuretics" by K. H. Beyer, Jr.

The other topics are of equal interest and timeliness. In addition, there are appended cumulative indices of the chapter titles and authors for all 11 volumes of the series.

C.Z.

Toward the Diagnosis of Congenital Heart Disease. By W. Carleton Whiteside, M.D. Pp. 86. Springfield, Ill.: Charles C Thomas, 1960. Price, $4.50.

Of the many ways in which a book is finally composed, this one is unique because it is in outline form, purposely, to permit simple presentation of information which, whether rapidly digested or retained, might be sufficient, the author hopes, for correct diagnosis and stimulation of continued interest. A potpourri of various aspects and types of congenital heart disease is given, following an introduction consisting of some 136 diagnostic principles. The book is concluded with do's and don'ts regarding cardiac arrest. For spicing, there is added interesting quotations, some philosophical and others in the category of "pearls." Of these, one expresses succinctly the author's desire for those who read his book:

"Adventure on, for from the littlest clue
Have come whatever man ever knew."

J.Z.

The Arterial Wall. Edited by Albert I. Lansing, A.B., Ph.D., Chairman, Department of Anatomy, University of Pittsburgh School of Medicine. Pp. 259; 18 ills. Baltimore: The Williams & Wilkins Company, 1959. Price, $7.50.

This volume undertakes to review the data that are currently available on the structure, function, and chemistry of the major components of the arterial wall. Chapters are devoted to the following topics: "Vasa Vasorum of Arteries," "Structure and Behavior of Vascular Endothelium," "Perspectives in the Study of Arterial Muscle," Collagen and Ground Substance," "Elastic Tissue," Mucopolysaccharides of Arterial Tissue," Enzymes of Arterial Tissue," Lipid Metabolism of Connective Tissue as Related to Vascular Aging," and "The Metabolism of the Arterial Wall."

The contributions are all well-written, objective, and maintain a broad perspective. There is much here to interest those engaged in the investigation of atherosclerosis. C.Z.

Fundamentals of Nutrition. By E. W. Crampton and L. E. Lloyd, McGill University. Pp. 494; 46 ills. San Francisco: W. H. Freeman and Co., 1960. Price, $7.50.

This is a text written for the serious student of nutrition providing a superb integration of basic facts of the nature of nutrients and metabolism with the functioning of tissues and their nutritional needs. In the first of five sections of the book, the nutrients, their properties and proximate analysis by the conventional Weende method are described. The metabolism of water, composition of animal tissues and the physiology of digestion are included in the introductory section. In the second section, the nutrients are traced through the metabolic pathways of cells and are placed in their appropriate roles in the nutritive economy of the body. The intermediary metabolism of fats, protein and carbohydrate are considered individually and in relation to the final common pathway of energy metabolism. These processes are expertly correlated with the catalytic and regulatory mechanisms contributed by vitamins and cofactors discussed in the third and fourth sections. Throughout the text are found numerous diagrams and charts which facilitate comprehension of these nutritional concepts. The book is a very valuable contribution to a field in which basic material is frequently ignored in teaching of the subject. C.S.

Lectures on Haematology. Edited by F. G. J. Hayhoe, M.D., M.R.C.P., Department of Medicine, University of Cambridge. Pp. 247; 73 ills. New York: Cambridge University Press, 1960. Price, $11.50.

This volume consists of a series of lectures delivered at a Cambridge Postgraduate Medical Course held in December, 1959. The lectures are informative reviews of important topics in hematology, given by well-known authorities who have themselves contributed significantly to their respective fields. A partial list of the subjects discussed includes: Acquired Haemolytic Anemia (by J. V. Dacie); Iron Metabolism and Iron-deficiency

Anaemia (by W. M. Davidson); The Pathogenesis and Differential Diagnosis of Megaloblastic Anaemias (by R. H. Girdwood); Haemoglobin Variants (by J. C. White); Clinical and Cytological Recognition and Differentiation of the Leukaemias (by F. G. J. Hayhoe); Chemotherapy of Leukaemia (by D. A. G. Galton); and The Pathogenesis of Purpura (by J. F. Ackroyd).

Although the lectures are oriented primarily towards consultants and research workers who have a major clinical or laboratory interest in hematology, internists, pediatricans, pathologists and workers in other areas will find much of value in these excellent discussions.

C.Z.

AIDS TO NEUROLOGY. By E. A. BLAKE PRITCHARD, M.A., M.D., F.R.C.P., Physician in Charge, Department of Neurology, University College Hospital, London. 2nd ed. Pp. 480; 39 ills. London: Bailliere, Tindall and Cox, 1959 (The Williams & Wilkins Co., Baltimore, U.S. Agents). Price, $4.00.

THIS book according to the preface is designed to "provide an introduction to the basic essentials of neurology for students in the earlier years of their general medical training." This it does, although perforce, it is brief. Even at that, it fills a need in the scheme of the busy student who cannot find the time for more complete treatises.

The material is presented from a descriptive standpoint including syndromes of the peripheral nervous system, the spinal cord, the brain and the vegetative nervous system. Cause and treatment are also included in addition to some explanation of symptoms in terms of physiology and anatomy.

As with all books from overseas, in the experience of this reviewer, there are some differences from the generally accepted concepts held in this country. The therapy of myasthenia gravis was too briefly considered. The larger dosage of Vitamin B_{12} in pernicious anemia with neurological involvement is not mentioned and this would seem to be important. English names for drugs are not accompanied by the American equivalents which would be quite helpful. The very brief space given to "the Clinical Examination of Nervous Activities" is scarcely worth while. The short discussion of the electro-encephalogram is excellent for a book of this size.

Finally, looking back to his medical school days, this reviewer must admit that he would have appreciated such a summary in his course in neurology.

S.G.

THE LYMPHOCYTE AND LYMPHOCYTIC TISSUE. Edited by JOHN W. REBUCK, M.D., PH.D., Chief, Division of Laboratory Hematology, Henry Ford Hospital, Detroit. Pp. 312; 181 ills. New York: Paul B. Hoeber, Inc., 1960. Price, $10.50.

THIS is the first of a projected series of volumes sponsored by the International Academy of Pathology. It consists of material from the course on "The Lymphocyte and Lymphocytic Tissue" which was moderated by Dr. Rebuck in 1959. There are 21 contributing authors, all highly qualified to discuss their respective topics, a partial list of which follows: Lymphocytes: Origin, Structure, and Interrelationships (by Dorothy Sundberg); The Embryogenesis of Lymphocytic Tissue (by W. Bloom); Cytochemistry of the Lymphocytes (by G. A. Ackerman); Electron Microscopy of the Lymphocyte (by F. N. Low); Lymphocytic Life Span and Turnover (by J. L. Bollman); Role of the Lymphocyte in Antibody Formation (by J. C. Roberts, Jr.); The Changing Pattern of Lymphocytic Malignancies (R. P. Custer); and Structure of the Lymphocytic Series of Cells in Relation to Disease (by J. W. Rebuck).

Hematologists, pathologists, and others interested in the lymphocytic elements will find this a most valuable reference source. The editor and his contributors are to be commended for the over-all excellence of this volume. They have set a high standard for conference reports which are to follow.

C.Z.

HANDBOOK OF NEUROLOGICAL DIAGNOSTIC METHODS. Edited by FLETCHER MCDOWELL, M.D., Assistant Professor of Medicine (Neurology) AND HAROLD G. WOLFF, M.D., Professor of Medicine (Neurology), Cornell University Medical College. Pp. 201; 65 ills. Baltimore: The Williams & Wilkins Co., 1960. Price, $4.50.

IT is seldom that this relatively unemotional reviewer comes on a book which calls forth such unrestrained approval as this short discussion of methods used in neurological diagnosis elicits. From his experience of some years this should be required reading and study for all students of medicine. Internes and residents will voluntarily recognize its value and consult it. Nowhere else is this information available in such short, concise fashion and most important to the young medical man, at such a reasonable price. This last is, of course, a recognized attribute which all medical men know only too well.

Included are discussions of how to elicit a history with details which all of us know from experience but which are so seldom put into print. Also emphasized is the emotional or psychologic element and this in a neurological treatise! This deserves emphasis as all know but too often the organic and nonorganic are considered separately to the consequent detriment of the patient at times.

In summary this reviewer knows from fairly long experience at trying to teach neurology that in this little book the student will find that for which he has been looking. The recommendation will be made by him that all who can should have the book and it should be given the same consideration as the reflex hammer and the tuning fork, a must for which sacrifice should be made. S.G.

THE DISPENSATORY OF THE UNITED STATES OF AMERICA. By ARTHUR OSOL, PH.G., B.S., M.S., PH.D., Professor of Chemistry and Dean of Science, Philadelphia College of Pharmacy and Science AND ROBERTSON PRATT, A.B., PH.D., Professor of Pharmacognosy and Antibiotics, University of California School of Pharmacy. 2nd ed. Pp. 240; 127 ills. Philadelphia: J. B. Lippincott Company, 1960. Price, $9.00.

VOLUME Two of the United States Dispensatory admirably fills the need for a single, reliable source of information about new pharmacologic agents developed since publication of Volume One five years ago. It contains as well much new information concerning some agents contained in Volume One. Volume Two is "The New Testament" of "The Bible" on drugs, their chemistry, pharmacology and clinical usage. W.S.

LYMPHOCYTES AND MAST CELLS. By MARGARET A. KELSALL, M.A., PH.D., Research Staff (Biology) Research Service Laboratories, University of Colorado, AND EDWARD D. CRABB, M.A., PH.D., Professor of Biology, University of Colorado. Pp. 399. Baltimore: The Williams & Wilkins Co., 1959. Price, $8.00.

THERE is much basic information regarding trephocytes (feeding cells) which has been brought together in this correlative review. Trephocytes are cells which exist primarily for the benefit of other cells and exhibit functions that include supply of nutritive materials and regulation of protein metabolism. The present work is devoted to a systematic consideration of the trephocytic activities of lymphocytes, plasmocytes, and mast cells. The following topics are discussed in successive chapters: Lymphocytes and Plasmocytes; Mast Cell Interrelationships; Physiological Distribution of Mast Cells; Histamine; Effect of Mast Cell Products on Capillary Permeability; Heparin and Hyaluronic Acid; Thymus; Spleen; Bone Marrow; Intestinal Lymphoid Tissue Relationships; and Inflammation and Wound Healing.

This volume will be of value not only to biologists and biochemists but also to hematologists and others who are concerned with the same or related areas of investigation.

C.Z.

COMPLICATIONS IN SURGERY AND THEIR MANAGEMENT. By CURTIS P. ARTZ, M.D., F.A.C.S., Associate Professor of Surgery, University of Mississippi, and JAMES D. HARDY, M.D., F.A.C.S., Professor and Chairman, Department of Surgery, University of Mississippi. Pp. 1,000; 270 ills. Philadelphia: W. B. Saunders Company, 1960. Price, $23.00.

THIS book is a new approach in surgical writing and is an exhaustive organized listing of complications in surgical patients. Each section has been written by an individual and there are some seventy authors contributing to this text. Many of these authors are well known in American Surgery today. Many of the complications that are general to surgery are treated repetitiously by the separate authors and although the editors have apparently tried to keep this at a minimum, it has not been eliminated. The text is adequately supplied with an index and detailed list of contents with adequate illustrations. I believe this book to be particularly valuable to the resident physician as well as to the young surgeon who has had limited experience in some of the fields. Certainly this is the only single source where one can find a listing of possible complications, their recognition and suggestions as to prevention or treatment. R.T.

ANNUAL REVIEW OF BIOCHEMISTRY, VOL. 29, 1960. Edited by J. M. LUCK, F. W. ALLEN AND G. MACKINNEY. Pp. 786; illustrated. Palo Alto, Calif.: Annual Reviews, Inc., 1960. Price, $7.00.

THE present volume is the 29th in this series of reviews of developments in selected areas of biochemistry. It opens with an inter-

esting, personal chapter by the late H. O. L. Fischer entitled "Fifty Years 'Synthetiker' in the Service of Biochemistry." Among the topics reviewed are: Mechanism of Enzyme Action (by P. D. Boyer); Glycolipids (by J. H. Law), Chemistry of Amino Acids and Peptides (by R. Schwyzer); Lipid Metabolism (by P. K. Stumpf); Nutrition (by M. O. Schultze); The Biochemistry of Genetic Factors (by R. L. Sinsheimer); Biochemistry of Cancer (by E. P. Anderson and L. W. Law); and Immunochemistry (by P. Haurowitz).

These and the other chapters are informative and invaluable as references. A Cumulative Index of Chapter Titles for Volumes 21 to 29 is appended. C.Z.

Lipids and the Steroid Hormones in Clinical Medicine. Edited by F. William Sunderman, M.D., Ph.D., Sc.D., Director, Division of Metabolic Research and Clinical Professor of Medicine, Jefferson Medical College, and F. William Sunderman, Jr., M.D., Head, Chemistry Branch and Instructor in Clinical Pathology, U.S. Naval Medical School, Bethesda, Md. Pp. 207; illustrated. Philadelphia: J. B. Lippincott Co., 1960. Price, $10.75.

This volume records the Proceedings of an Applied Seminar of the Association of Clinical Scientists. Part I is devoted to the Lipids, with general considerations, methods of determination, and clinical significance of various parameters discussed by experts in the respective areas. A similar treatment is given to The Steroid Hormones in Part II.

Clinical investigators, clinical pathologists and biochemists, internists, endocrinologists and others working in lipid metabolism will find this a particularly valuable guide to the selection of reliable analytical methods and for the interpretation of their results. C.Z.

Year Book of Medicine, 1960-1961 Series. Edited by Paul B. Beeson, M.D., Carl Muschenheim, M.D., William B. Castle, M.D., Tinsley R. Harrison, M.D., Franz J. Ingelfinger, M.D., and Philip K. Bondy. Pp. 740; 108 ills. Chicago: Year Book Publishers, Inc., 1960. Price, $8.00.

The current volume of this Year Book consists of abstracts of a large number of articles which appeared during the "Series Year." The material reviewed is presented in 6 general sections: 1) Infections; 2) The Chest; 3) The Blood and Blood-Forming Organs; 4) The Heart and Blood Vessels and the Kidney; 5) The Digestive System; and 6) Metabolism.

The abstracts are brief but sufficiently comprehensive to be informative. Editorial annotations enhance the over-all value of the volume. C.Z.

Probleme der Blutgruppenforschung. By Prof. Dr. Ludwik Hirszfeld. Pp. 160; 29 ills. Jena: Gustav Fischer Verlag, 1960. Price, 22 DM.

The serologic aspects of blood groups, antibody structure, genetic aspects of various blood factors, immunopathology of pregnancy, the chemistry of blood group substances, and the anthropologic implications of blood groups are among the topics discussed in this small monograph.

It may be recommended as a practical guide to students and physicians who read German. C.Z.

Genetics. Edited by H. Eldon Sutton, Ph.D., Department of Medical Genetics, University of Michigan Medical School. Pp. 229; 38 ills. New York: The Josiah Macy, Jr., Foundation, 1960. Price, $6.00.

The transactions of the First Conference on Genetics held in 1959 under the auspices of the Josiah Macy, Jr. Foundation are published in this volume. Three major areas were discussed, namely: Current Concepts of the Fine Structure of Chromosomes and the Nature of the Coding Mechanism (J. D. Watson); The Genetic Control of Protein Structure (V. M. Ingram); and Factors Modulating the Biochemical Expression of Genetic Systems (S. Spiegelman). The comments of other participants and special summaries are also recorded, thereby enhancing the over-all value of the book.

This volume, along with the future symposia of this series, should provide an authoritative, interdisciplinary delineation of genetic information and the control of protein structure and function. C.Z.

Biochemistry of Human Genetics. Edited By G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch. and Cecilia M. O'Connor, B.Sc. Pp. 347; 60 ills. Boston: Little, Brown and Co., 1959. Price, $9.50.

This volume contains the proceedings of a Symposium on "Human Biochemical Genetics

in Relation to the Problem of Gene Action," held in 1959. The meeting was well-organized and, after an introduction to genetical analysis in Man, the first section was devoted to some areas of genetic control of metabolism. The second general area of discussion was concerned with the synthesis of proteins and other macromolecules, while the third section dealt with basic facts relevant to gene action.

Twenty-nine outstanding scientists contributed to this symposium and produced an exceptionally informative and valuable contemporary orientation in human genetics. It is recommended not only to those whose fields of endeavor are related to genetics but also to all physicians who are students of medicine. C.Z.

TRANSPORT AND ACCUMULATION IN BIOLOGICAL SYSTEMS. By E. J. HARRIS, PH.D., D.I.C., D.Sc., Department of Biophysics, University College, London. 2nd ed. Pp. 279; 88 ills. New York: Academic Press, Inc., 1960. Price, $9.00.

RAPID advances since the first edition have led to an almost complete rewriting of this treatise on the kinetics and mechanisms of movement of some simple cellular constituents between cells and their surroundings. The discussion is at a basic level, with a general consideration of the factors which govern the distribution and movement of water and ions, respectively. There are also numerous specific examples of the mechanisms involved in certain cells or cellular components.

Investigators concerned with physicochemical transport mechanisms at the cellular level will find this a valuable reference work. C.Z.

DISEASE AND THE ADVANCEMENT OF BASIC SCIENCE. Edited by HENRY K. BEECHER. Pp. 416; illustrated. Cambridge: Harvard University Press, 1960. Price, $12.50.

THE purpose of this volume, which developed from the Lowell Lectures of 1958, is to show that some kinds of fundamental advance in science can be made only from study of disease. Illustrative chapters were contributed by 21 distinguished scientists, including W. B. Castle, C. F. Cori, L. Pauling, W. Bauer, R. W. Gerard, P. Bailey, J. H. Means, F. D. Moore, H. K. Beecher, and R. J. Dubos.

The presentations are interesting, informative, and stimulating. All serious students of clinical medicine will find this book to be highly rewarding. C.Z.

CARCINOGENESIS: MECHANISMS OF ACTION. Edited by G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., B.CHI., AND MAEVE O'CONNOR, B.A. Pp. 336; 48 ills. Boston: Little, Brown and Co., 1959. Price, $9.50.

A symposium on Carcinogenesis: Mechanisms of Action was held under the auspices of the Ciba Foundation in 1958. The presentations and the accompanying discussions of that meeting have been recorded in this volume. All of the papers are fundamental in their approach and offer worthwhile reading for workers engaged in cancer research. C.Z.

THE MICROCIRCULATION. Edited by S. R. M. REYNOLDS AND BENJAMIN W. ZWEIFACH. Pp. 170; 72 ills. Urbana, Ill.: University of Illinois Press, 1958. Price, $4.50.

THIS volume contains the proceedings of the Fifth Conference on Microcirculatory Physiology and Pathology which was held in Buffalo, New York, in 1958. Factors which influence the exchange of substances across the capillary wall served as the theme for this symposium. Among the topics discussed were: The Fine Structure of Capillaries, Arterioles, and Small Arteries; Capillary Permeability and Transcapillary Exchange in Relation to Molecular Size; Macromolecular Exchanges in Capillaries; Properties of Artificial Membranes with Microscopic Pores; A Study of Lymphatic Permeability to Albumin; and Structural and Functional Aspects of the Microcirculation in the Skin.

Anatomists and physiologists will find the presentations and the comments concerning them to be highly informative with regard to these minute blood vessels. C.Z.

THE FUNDAMENTALS OF NERVE BLOCKING. By VINCENT J. COLLINS, M.D., Associate Professor of Anesthesiology, New York University Medical Center, and Anesthesiologist at Bellevue Hospital Center, with the assistance of EMORY ANDREW ROVENSTINE, M.D., Professor of Anesthesiology and Chairman, Department of Anesthesiology, New York University Medical Center. Pp. 354; 144 ills. Philadelphia: Lea & Febiger, 1960. Price, $9.50.

This small monograph is divided into two parts. Part A: Principles and Problems, Part B: Regional Techniques. Part A contains 16 small chapters and Part B, 8 chapters.

The book is well organized. The material is succinctly presented and the illustrations are excellent. It will be valuable to all utilizing nerve blocking techniques.

The author has not stressed the possibility of aggravation of pain following alcohol injection. He leaves the assumption that the relief of pain following a nerve block is always the result of the successful injection of anesthetic into the nerve or nerve pathway. Although this is usually so pain complaints can be occasionally relieved completely by suggestion plus, for example, the production of small intradermal wheals with saline. In other words the pain can be stopped by "suggestion and the laying on of hands." This ability of the operator to "reverse" pain complaints through the use of injections with or without suggestion must always be evaluated in deciding the true value of a nerve block procedure. M.S.

Radioisotope in der Herzdiagnostik. By Priv. Doz. Dr. med. Hans Ludes and Dr. med. Gerhard Lehnert, Medical University Hospital, Cologne. 2nd ed. Pp. 120; 67 ills. Jena: Gustav Fischer Verlag, 1960. Price, DM 16,90.

The application of radioisotopes in cardiac diagnosis is based upon three principles: dilution in the blood for determination of blood volume and heart minute volume; transportation of radioactive indicator in vascular system to determine circulation time; and inflow and outflow measurements of the local circulatory condition in the heart muscle. In the problem of congenital abnormalities, both dilution and transportation methods have been applied. Isotope angiography offers much less risk of radiation compared with regular angiocardiography. When reading the chapter on dosimetry and radiation protection, one would like to have a physicist over one's shoulder. The chapters do not offer summarizing, partly because of the flux of developments and partly because the methods and critical evaluations are presented sequentially. The available amount of knowledge is enormous, a few practical applications are already in use, all of it is of importance for research. The references, predominantly of U.S.A. and German contributors, would seem complete, 223 entries are noted. Significant illustrations from the literature are clearly presented. H.R.

Cerebral Palsy and Related Disorders: The Developmental Approach to Dysfunction. By Eric Denhoff, M.D., Clinical Director, Meeting Street School, Children's Rehabilitation Center, Providence, Rhode Island, and Isabel Pick Robinault, Ph.D., Director, Professional Education Institute for Crippled and Disabled, New York City. Pp. 421; 21 ills. New York: McGraw-Hill Book Co., Inc., 1960. Price, $12.00.

This work introduces a new concept that growth and development are an ever-changing facet in the lives of children who are handicapped with cerebral palsy, epilepsy, mental retardation of organic origin and hyperkinetic impulse disorders.

These authors have studied how these children behave and how they react to their environment. The book is divided into 12 chapters and includes an appendix for films for professional use, a name index, and an institution index. The authors attempt to demonstrate how physicians and other professional personnel in any area can realistically handle problems of handicapped children by using treatment from the functional approach rather than in the light of fixed disability.

This monograph presents a logical and practical approach and incorporates in a distilled manner, recent concepts as to psychological, surgical, and rehabilitation therapy. This interest in cerebral palsy is widespread and covers every facet of medicine. This monograph should be read by all interested in this problem. M.S.

The Misrepresentation of Arthritis Drugs and Devices in the United States. A report to the Committee on Arthritis Advertising, the Arthritis and Rheumatism Foundation. By Ruth Walrad, Research Consultant. Pp. 168. New York: The Arthritis and Rheumatism Foundation, 1960. Price, $3.50.

Sufferers from various forms of arthritis and rheumatism in the U.S.A. are, each year, deceived into paying approximately 252 millions of dollars for pretended cures or "spectacular" relief of their symptoms. This book surveys spurious products so advocated; discusses fully the growth and development of controls of such mispresentation and makes recommendations for improvement of the present situation. Four appendices deal with; State laws on labeling and advertising; Accepted Advertising Practice and Television codes, and others. A selected bibliography and an Index conclude the volume. This should be a most useful source book for anyone concerned

with curbing the peddlers of false hope who callously enrich themselves at their victim's expense. J.L.

The Chemistry of Thyroid Diseases. By Rosalind Pitt-Rivers, M.Sc., Ph.D., and Jamshed R. Tata, M.Sc. Pp. 83; 10 ills. Springfield, Ill.: Charles C Thomas, 1960. Price, $4.50.

The biochemical phenomena which underlie various aspects of normal and abnormal thyroid function are discussed in this small monograph. It should be of interest not only to biochemists but also to clinical investigators who wish a summary of current information on the chemistry of the thyroid gland. C.Z.

Outline of Pathology. By John H. Manhold, Jr., D.M.D. and Theodore E. Bolden, D.D.S., College of Dentistry, Seton Hall College of Medicine and Dentistry and Jersey City Medical Center. Pp. 340. Philadelphia: W. B. Saunders Co., 1960. Price, $4.75.

This book represents an outline of Pathology and as such consists mainly of classifications of pathological processes and their definitions. Actually, the sole aim of the authors was to produce a text, which would be brief and concise and which would serve as a guide for studying and reviewing the subject of Pathology. The authors have accomplished their purpose. A.P.

The Liver and Cancer. By Kasper Blond, M.D., F.I.C.S., 2nd ed. Pp. 400. Bristol: John Wright and Sons Ltd. (The Williams & Wilkins Co., Baltimore, exclusive U.S. agents). Price, $8.00.

This is an interesting book depicting the concept of the author relative to the cause of cancer. Briefly stated Dr. Blond postulates that cancer is the end phase of chronic nutritional disturbance resulting from liver damage. He considers chronic disease as a "precancerosis." The prevention of cancer according to his theory depends on correct nutrition. Dr. Blond is dogmatic in his views and criticisms, and as stated in the foreword he impresses one as an "original thinker" and as a "rebel against blind orthodoxy." A.P.

Biochemical Aspects of Microbial Pathogenicity. By Werner Braun and D. V. Siva Sankar and 44 Others. Pp. 297; illustrated. New York: New York Academy of Sciences, 1960. Price, $5.00.

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